Dissecting genetic variation for nitrogen use efficiency in wheat

A thesis submitted for the degree of Doctor of Philosophy

by

Saba Mahjourimajd

Supervisors:

Prof. Peter Langridge

Dr. Mamoru Okamoto

Dr. Haydn Kuchel

Faculty of Sciences

School of Agriculture, Food and Wine

Discipline of Plant Breeding and Genetics

Australian Centre for Plant Functional Genomics

THE UNIVERSITY of ADELAIDE

May 2015

Table of Contents

Thesis Abstract

Nitrogen (N) is essential for high grain yield (GY) in cereals. A major aim of breeding programs is to increase GY while minimising the level of external inputs, such as N fertilisation. Nitrogen Use Efficiency (NUE) is a complex trait controlled by both genetic and environmental factors resulting in variation depending on seasonal growth conditions. Only 30-50% of N supplied is actually taken up by the plants with the extra N lost through run-off, leaching, denitrification and gas emission. These losses have a negative environmental impact, leading to surface and underground water pollution, algae blooms and intensifying global warming. In addition, nitrogen (N) application is costly further emphasising the importance of NUE improvement to reduce the economic and environmental issues associated with N application. NUE of wheat is important in all production areas but little is known about genetic variation for NUE in low-yielding environments such the Mediterranean-type climate of Southern Australia with low rainfall and high temperatures during critical growth periods. Research described in this thesis evaluated variation in NUE in Australian wheat germplasm and then to identify loci regulating NUE traits in a bi-parental mapping population of RAC875/Kukri. Improvement in NUE will require the integration of physiological and molecular aspects of N status in plants under different growth conditions: the highly variable conditions of field trials and controlled environments such as under hydroponics. The assessment of NUE and N response under both field and controlled conditions could facilitate the identification of traits and QTL and lead to the discovery of candidate genes underlying the traits.

The first step of this research involved NUE traits and N response assessment of Australian cultivars in different environments, with varying N input. Genetic variation for NUE was identified in Australian spring wheat cultivars, and the cultivars were ranked for their N-efficiency and responsiveness. The dissection of genetic variation for NUE was investigated in the RAC875/Kukri population across six field trials between 2011 and 2013 covering 16 environment by treatment combinations. Nitrogen responsiveness was compared

with N efficiency and the genotypes were ranked for the consistency of a positive response and high efficiency of N use versus negative responsiveness and low efficiency. Quantitative Trait Loci (QTL) analysis identified the genome regions associated with GY, grain quality and responsiveness to N. In addition, specific-environment associated N QTL were identified. A QTL on chromosome 2A was detected for most of traits studied and across multiple environments. Further stable QTL were identified on chromosomes 1A, 1B, 2A, 3D, 7A and 7B for GY across environments. The physiological response to N was studied at the early stages of growth for selected lines in a hydroponics system that allowed the measurement of N uptake and utilisation. The aim of the experiments was to investigate the physiological basis for the effects seen in the field trials. However, no consistent response was seen in these studies suggesting that future work should focus on later growth stages.

To conclude, the results showed significant genetic variation and transgressive segregation for NUE despite the complex nature of the effect of N on grain yield and quality traits. These genome regions can be used to support marker assistance selection (MAS) for improved NUE and for cloning genes underlying the loci affecting NUE in wheat. The results show that selection for improved NUE is possible and also provide a base for further molecular and physiological studies on efficient use of applied N.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

The author acknowledges that copyright of published works contained within this thesis resides with the copyright holder(s) of those works.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library catalogue and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Saba Mahjourimajd

December 2014

Acknowledgements

I would like to use this opportunity to express my gratitude to all people who helped and supported me throughout my thesis. First of all, I am deeply thankful to Peter, my principle supervisor, for all his guidance, invaluable comments and brilliant views on many issues related to my Ph. D. project and his friendly encouragements during my work. It definitely was his support that made me study and work enthusiastically at the Australian Centre for Plant Functional Genomics (ACPFG) and the University of Adelaide and drove me to think of new perspectives in plant breeding. I learned lots of scientific and life lessons from him and I am honoured to have been one of his students. My warm thanks to Mamoru, for his kind and patient support and helpful suggestions. I am sincerely grateful to Haydn, for his guidance and his great analytical ideas for interpreting the results of experimental tests.

Thanks to Julian Taylor who assisted me in statistical analysis of the data and thanks also to Glen McDonald, my independent advisor, Ken Chalmers, my postgraduate coordinator, Monica Ogierman, my previous education manager and Ruth Harris, my English tutor for their patiently and positively support and encouragement.

I am sincerely grateful for having a chance to meet so many wonderful people and professionals including supervisors, students, lab managers and Administrative people at the ACPFG who provided kind cooperation and encouragement to enjoy every moment of my study. Also, special thanks to the University of Adelaide and ACPFG manager which provided me with full-scholarship and all required facilities and conducive conditions to do my Ph. D in Australia.

I would also like to thank all organisations including the Australian Grain Technologies team at Roseworthy, barley group, SARDI group at Waite campus, Geranium people at Lameroo, technicians, casuals and everybody who helped me to provide this dissertation.

Last but not least, my great thanks and appreciation to my family, particularly my Mum as my first teacher, for all their support and inspiration throughout my life and education.

Abbreviations

N	: Nitrogen
NUE	: Nitrogen Use Efficiency
GY	: Grain Yield
RGY	: Responsive Grain Yield
GPC	: Grain Protein Concentration
PY	: Protein Yield
DH	: Doubled Haploid
QTL	: Quantitative Trait Loci
LOD	: Logarithm of the odds
G×E	: Genotype-by-Environment Interaction
SR	: Seeding Rate
BLUP	: Best Linear Unbiased Predictors
NO_3^-	: Nitrate
NH_4^+	: Ammonium
HATS	: High Affinity Transport System
LATS	: Low Affinity Transport System
^{15}N	: Labelled Nitrogen
HN	: High Nitrogen
LN	: Low Nitrogen

Chapter 1

General Introduction

Bread wheat (*Triticum aestivum* L.) is the third largest crop in production, the second most important food crop after rice, meeting 20% of the global human protein demand and the most widely grown crop. With a predicted world population of 9 billion in 2050, the demand for wheat is expected to increase by 60%. To meet this demand wheat yield needs to increase by 1.6% annually (FAO 2012).

Many factors limit grain yield (GY) including the availability of nitrogen (N). The global rate of consumption for N fertiliser is higher than for other nutrients since it accounts for 62% of all fertiliser application (FAO 2011). In addition, studies showed that crops and cereals can take up only 30-50 % of N supplied and the remainder is lost (Craswell and Godwin 1984; Hodge et al. 2000). Therefore, the cost of N production and provision as a critical issue has persuaded plant breeders to improve varieties for better use of N and higher nitrogen use efficiency (NUE) in order to attain higher GY.

Generally, NUE is defined as the ratio of GY to N supplied and can be further dissected into its subcomponents of N uptake and N utilisation efficiency. Both components are controlled by genetic and environmental factors (Gallais and Coque 2005; Laperche et al. 2006b; Coque et al. 2008). In this research NUE was assessed as the response of genotypes to N application for GY, quality parameters and related traits. In the following chapters, NUE is evaluated from four different perspectives. In Chapter 3, "Evaluation of Australian wheat genotypes for response to variable nitrogen application" is presented. This study was an assessment of genetic variation and stability of N response by wheat cultivars in low-yielding areas of South Australia.

Quantitative trait loci (QTL) analysis has been one of the best approaches to identify the genome regions underlying quantitative traits (Collard et al. 2005). This has been applied to the dissection of NUE in crops such as maize (Hirel et al. 2001; Gallais and Hirel 2004), rice (Obara et al. 2004; Lian et al. 2005; Cho et al. 2007) and wheat (Laperche et al. 2007; Fontaine et al. 2009; Bogard et al. 2011; Cormier et al. 2013; Xu et al. 2014). In Chapter 4,

“Genetic basis for variation in grain yield under different nitrogen amounts in wheat**”** and Chapter 5 “The genetic control of grain protein content under variable nitrogen supply in an Australian mapping population” QTL analysis was employed to better understand the genetic background of N response in a sub-population derived from a cross between two popular Australian cultivars, RAC875 and Kukri. Grain yield and protein-related traits along with N-associated characteristics were included in the analyses. Significant genome regions underlying GY and protein components were detected in the population.

Further, in Chapter 6, “An investigation of nitrogen response in Australian genotypes under field and controlled conditions” the physiological basis of N response in wheat genotypes selected in the field trials was studied under hydroponics culture and controlled environmental conditions.

Chapter 2

Literature Review

2.1 Introduction

Nitrogen (N) is the major component of nucleotides and proteins in living organisms. N fertilisation plays an important role in agriculture through its effects on grain yield (GY) and/or total biomass in crops including wheat. However, N fertiliser production is energy demanding and therefore expensive, and excessive N application has negative environmental consequences. Consequently, N fertiliser needs to be applied over the life cycle of the plant to optimise growth and nitrogen use efficiency (NUE) while reducing the cost of application and environmental pollution (Robertson and Vitousek 2009).

In this chapter, NUE and methods for its improvement are reviewed as a background to the experimentation contained within this thesis.

2.2 The role of nitrogen (N) in plants

Adequate N rates are essential for efficient use of N fertiliser and to maintain the economic sustainability of cropping systems. There is a high N concentration in plants during vegetative growth period then it reduces due to slower N assimilation (Gasser and Thorburn 1972; Gregory et al. 1979). However, uptake N from the soil continues to maturity. In addition, wheat cultivars with a large biomass at anthesis could uptake less N post anthesis under low N availability in the soil (Cox et al. 1985). The N concentration in leaf tissue is also significantly higher than that of the stem throughout wheat growth indicating that N translocation from leaves occurs prior to accumulation in the stem (Harper et al. 1987). These N mechanisms can affect N allocation and the efficiency and response to N in plants and this will govern GY and grain protein concentration (GPC) (Hirel et al. 2007).

2.2.1 Grain yield (GY)

Increasing N fertilisation increases yield components such as harvest index and kernels per spike, and chlorophyll content during the growth season leading to enhance GY in wheat crops (Cormier et al. 2013; Wang et al. 2014; Xu et al. 2014). Previous research has shown

that there is genetic variation for GY at varying levels of N supply (Laperche et al. 2007; Bordes et al. 2013). In contrast, excessive N supply in wheat fields could affect negatively NUE, GY and its components by the elevation of NO_3^-N concentration in groundwater (Wang et al. 2011) and haying-off (low or negative GY responses to increasing N application, McDonald 1992). Management of N application should be considered to achieve optimum GY and NUE while avoiding N loss.

2.2.2 Grain Protein Concentration (GPC)

Grain protein Concentration (GPC) is an important criterion of wheat grain quality and therefore a major driver for the grading of grain. Because N is one of the key building blocks of protein, GPC is influenced by both the amount and timing of N application. There are many studies showing increased protein concentration in grain at high N level with genetic variation for GPC (Charmet et al. 2005; Bogard et al. 2011; Bordes et al. 2013). Bogard et al. (2011) studied GY and GPC with varying N treatments and identified some genomic regions underlying protein composition on chromosomes 2A, 2D, and 7D in wheat. They confirmed the negative genetic correlation between GY and GPC which hampers the concurrent genetic improvement of these traits. Tindall et al. (1995) evaluated N applications at heading in irrigated hard red spring wheat in a three-year study. The study showed an inconsistent response of GY with N applications at heading, but a consistent grain protein response. Further, N applications at heading resulted in a GY decrease but an increase in GPC. Le Gouis et al. (2000) also emphasised that higher GPC requires the translocation of N before maturity.

2.3 Nitrogen use efficiency (NUE) in plants

The main goal of plant breeding programs is to achieve higher productivity, which ultimately requires an optimisation of NUE (Raun and Johnson 1999). Nitrogen use efficiency is a complex trait governed by several genes which differs from genotype to genotype (Vansanford and Mackown 1986; Dhugga and Waines 1989; Ortiz-Monasterio et al. 1997;

Laperche et al. 2007). Improving NUE and its main components could lead to a lower demand for applied N and, consequently, lower cost of fertilisation and reduced environmental pollution (Raun and Johnson 1999; MacDonald et al. 2013). From this perspective, the integration of agronomic research, physiological exploration and genetic dissection should be considered in NUE improvement to achieve high GY and protein yield (PY) (Moll et al. 1982; Hirel et al. 2007). Generally, NUE is defined in agronomy as the quantity of grain produced per unit of N applied (Moll et al. 1982). However, from a physiological perspective, it is calculated based on two main components; N-uptake efficiency (NupE: total above-ground N/soil N supply) and N-utilisation efficiency (NutE: GY/total above-ground N) which are controlled by genetics, the environment and the interaction of these factors (Gallais and Coque 2005; Laperche et al. 2006b; Coque et al. 2008).

2.3.1 Nitrogen uptake

The first phase in the N pathway is vegetative, including N uptake and assimilation by the developing roots (Hirel and Lea 2001). The root characteristics, morphology and growth rate in crop plants are the main factors which lead to significant variation in NupE among cultivars. In addition, the genotypic variation for greater growth is an important factor associated with high N uptake rates. Liao et al. (2004) reported that plants with larger root growth demonstrated considerably higher root biomass and N uptake than plant with slow root growth. Previous studies showed that NupE is more strongly correlated with GY than NutE. For example, Dhugga and Waines (1989) conducted experiments on different varieties of spring and durum wheat crops. They suggested that to improve NUE, selection for NupE would be more useful than the selection for NutE. Further Gastal and Lemaire (2002) stated that the amount of N uptake depends on crop growth rate and N availability in the soil. They showed that N uptake reduces gradually following heading under field conditions. However, grain N content is mostly derived from N uptake by plants during grain filling after anthesis in favourable conditions (Tucker 2000). The variation for NupE and NutE depends on the level

of N application. Le Gouis et al. (2000) in a study of NUE in winter wheat demonstrated that the variation for NupE was higher than NutE at low level of N while it was the opposite at high levels of N supply.

Nitrate (NO_3^-) is, being more abundant than ammonium (NH_4^+), the most common form of available N for plants in temperate field conditions. (Xu et al. 2012). Uptake of both of these N forms is controlled by key enzymes and transporters; for example, ammonium transporter (AMT1) controls the uptake of NH_4^+ (Søgaard et al. 2009). In addition, nitrate uptake is governed by nitrate transporters families; NRT1, NRT2, and NRT3 (NAR2). Typically, two uptake pathways control nutrient uptake in plants; a high-affinity transport system (HATS) and a low-affinity transport system (LATS) (Glass et al. 2002). High-affinity transport system uptake system becomes apparent when transporters are active at low external nitrate concentration while LATS is active when the concentration exceeds (between ~ 200 to 500 μM).

2.3.2 Nitrogen assimilation

Once absorbed, plants convert nitrate and ammonium into amino acids and protein to use for cell function and development. The N assimilation starts with the reduction of nitrate to nitrite, and then to ammonium by nitrate reductase (NR) and nitrite reductase (NiR), respectively (Masclaux-Daubresse et al. 2010). The first reduction by NR takes place in the cytoplasm, and the second part of the assimilation by NiR, is localised in the plastids. Ammonium is assimilated into amino acids through the glutamine synthetase (GS)/ glutamate synthase (GOGAT) cycle in plastids. Glutamine synthetase is also localised in the cytoplasm (Xu et al. 2012). The GS/GOGAT cycle is also important to recycle ammonium from photorespiration and protein degradation. Nitrogen assimilation relies on the supply of adenosine triphosphate (ATP), ferredoxin (FDX) and nicotinamide adenine dehydrogenase (NADH) as products of photosynthesis, respiration and photorespiration. Studies have shown that GS activity is essential in N remobilisation, crop growth rate, increasing GY, and grain filling (Xu et al. 2012).

2.3.3 Nitrogen remobilisation

Nitrogen remobilisation and loading into the grain are the main components in NUE and N metabolism in plants since it influences seed production and GPC. Grain yield depends not only upon N uptake pre-anthesis but also on the reutilisation and remobilisation of N during grain filling hence the improvement of NutE causes better reutilised N from shoot into the grain (Kichey et al. 2007; Masclaux-Daubresse et al. 2008). Grain N content also affects germination efficiency and survival of young seedlings (Masclaux-Daubresse et al. 2010). Most of the grain N (60-95%) in wheat crops is provided by the remobilisation of stored N (Habash et al. 2007). However, the relative contribution of N remobilisation differs in crops, for example nitrate is used in most of crops, whereas ammonium is the N source in rice (Hirel et al. 2007).

To dissect the remobilisation step in crops, one useful method is apparent remobilisation in which the total N amounts at anthesis and harvest time are compared for an estimation of remobilised N (Masclaux-Daubresse et al. 2008). Joppa et al. (1997) and Mickelson et al. (2003) studied Quantitative Traits Loci (QTL) for N remobilisation and demonstrated the genes underlying the N metabolism pathway are apparently used for remobilisation in barley and durum wheat. Another method to monitor N remobilisation is a pulse-chase experiment with ^{15}N labelled N nutrient. Plants can be fed ^{15}N at certain stages of development, and the distribution of ^{15}N in the different parts of plant (i.e. leaf, stem, and grain) can be used at a later stage to estimate N remobilisation efficiency. ^{15}N-labelling experiments can also be performed in the field (Gallais et al. 2006). In some cases, the chlorophyll meter (SPAD) values of leaves can be used to predict N content in grain and therefore help growers decide the best time and quantity of N application (Lopez-Bellido et al. 2004).

2.4 Improving NUE

To improve NUE in crops, optimising N management and developing genotypes showing improved use of N in terms of efficiency and response has been recommended (Raun and

Johnson 1999). However, genetic studies in wheat can be difficult due to its polyploidy and the detection of a gene's expression is influenced by different environmental conditions (Chantret et al. 2005; Chen 2007). Unfortunately, there is still no generally accepted approach to attain high NUE in plants. However, there are many advances in studying N pathways and rates of N uptake, assimilation and remobilisation into the grain. Agronomic efficiency (AE, the ratio of [GY with N fertilistaion − GY without fertilisation] to N supplied) of N depends on the varieties and N provision levels (Guarda et al. 2004). This implies that there is an optimum level of N fertiliser to achieve AE and extra use of N fertiliser decreases efficiency. The genes encoding GS and GOGAT activity have been used in higher plants to evaluate N pathway. Previous studies demonstrated that increasing the activity of GS enhanced biomass and GY in plants (Good et al. 2004; Hirel et al. 2007; Lea and Azevedo 2007). A new molecular approach to improve NUE has been proposed based on over-expression or suppression of specific genes controlling main factors such as nitrate and ammonium transporters in transgenic plants (Good et al. 2004).

2.4.1 Agronomic perspective

Growers, through adjusting N application level and timing, can improve NUE. The amount of N required for optimum NUE varies in response to the quantity of residual N in the soil and the rainfall received during the current and previous seasons (López-Bellido et al. 1996). In crop estimates of N status, for example leaf chlorophyll content, can be used by growers to optimise their N application (Blackmer and Schepers 1994). This method was applied in wheat, combined with remote sensing technologies to estimate plant density and tiller number and to then calculate the amount of N to be supplied for attaining high GY (Flowers et al. 2003).

Fageria and Baligar (2005) listed soil chemistry modification, use of controlled release fertilisers and nitrification inhibitors, soil management, plant management and improving N fixation biologically and non-biologically (free-living micro-organisms or organisms not directly associated with higher plants are capable of non-symbiotic N fixation) as some of the

agronomic tools available to maximise NUE. Synchronising N plant demand and fertiliser application can also be used to achieve higher NUE and lower N losses (Goulding 2004; Peoples et al. 2004). In this view, increasing plant N demand, manipulating N supply, capturing the excess inorganic N before it is lost, information-intensive cultural adjustments, using technological innovations and pre-sowing soil testing for mineral N status could help us achieve the optimal concurrent control of N demand and supply (Crews and Peoples 2005). Crop rotation is another important strategy to reduce required N in crops. For example, N demand by plants is reduced when grown after legume crops since it allows efficient use of soil resources, especially nutrients and water (Gan et al. 2003).

2.4.2 Physiological perspective

To achieve high yield in low N supply, the selection for the physiological traits related to NUE would be beneficial (Blum 1988). The physiological factors affecting NUE include the source and sink balance at different stages of plant development (Bancal 2009), the critical N concentration in crops (Justes et al. 1994; Lemaire and Gastal 2009), biomass production (MacKown and Carver 2005; Greenwood et al. 2008), crop root system (Svoboda and Haberle 2006; Pedersen et al. 2010), GY and PY (Gulmezoglu and Aytac 2010). Hirel et al. (2001) studied some physiological traits to improve NUE such as nitrate content, NR, and GS in maize. The results demonstrated significant variation for these traits among the genotypes with a positive correlation with GY and its components except for NR. The authors hypothesised that NR and GS are key elements to improve NUE. In wheat, physiological N efficiency (PE, the ratio of [GY at N fertilisation – GY at no-fertilisation] to [N uptake at fertilisation – N uptake at no-fertilisation]) was measured by Guarda et al. (2004). They described that modern cultivars with higher GY and good NUE had increased PE. Moreover, Gallais et al. (2006) using a model of post-silking N fluxes demonstrated that a large proportion of N grain filling comes directly from post-silking N uptake in maize. Recently studied NUE-related factors include photosynthesis efficiency and Rubisco (ribulose 1, 5-

bisphosphate carboxylase) activity. These could improve NUE in plants and cereals through improving photosynthetic rate (Hibberd et al. 2008; Reynolds et al. 2009).

2.4.3 Genetic dissection

Genotypic differences in response of wheat to N fertiliser have been reported (Basso et al. 2010; Sadras et al. 2012). The heredity and genetic background of complex traits such as NUE can be studied using molecular markers to detect the loci underlying the traits. The markers showing strong linkage with the phenotype can be considered for genotypic selection in marker-assisted selection (MAS) (Agrama et al. 1999).

2.4.3.1 Quantitative traits loci (QTL) of NUE

By employing QTL approaches, researchers are able to identify chromosomal regions associated with particular quantitative traits. To do this, DNA markers are screened on segregating mapping lines which are phenotyped for the trait of interest. In the second step, the association between the expression of the trait and the inheritance of marker alleles is used to locate linked QTL. The location of QTL can be further refined with the inclusion of additional mapping data/lines; ultimately leading to the detection of candidate genes (Hirel et al. 2007). Rafalski (2002) reported that linkage disequilibrium can be applied to study large breeding populations from field trials. However, mapping populations derived from two contrasting parental lines have generally been used for NUE studies (Pestsova et al. 2000; Habash et al. 2007; Hirel et al. 2007; Le Gouis 2011; Xu et al. 2012). More recently, meta-QTL analysis has been used to combine multiple QTL mapping studies and focus on regions common across mapping populations and environments. In this way, loci associated with N response QTL can be more accurately mapped (Laperche et al. 2007).

An early QTL study targeting NUE and its related traits was performed in a mapping population derived from a cross between a local maize inbred line from Egypt and the US-Corn-Belt line B73 (Agrama et al. 1999). The lines were evaluated for yield under high and low N conditions over two years at one location in Egypt. Five QTL for GY under high N were detected on chromosomes 1, 4, 5, 9 and 10 and also six QTL under low N on

chromosomes 1, 2, 7, 9 (2) and 10. QTL were also detected for ear leaf area, plant height, kernels per ear, and kernel weight. Previous studies have reported finding QTL for NUE in Arabidopsis (Loudet et al. 2003), rice (Lian et al. 2005), maize (Agrama et al. 1999; Bertin and Gallais 2001; Hirel et al. 2001), barley (Kjaer and Jensen 1996) and wheat (An et al. 2006; Laperche et al. 2007; Bogard et al. 2011; Bordes et al. 2013). 17 QTL clusters for GY, across several trials containing strongest effects on chromosomes 7AL and 7BL have been detected in a doubled haploid wheat population derived from the cross Chinese Spring × SQ1 (a high abscisic acid-expressing breeding line) (Quarrie et al. 2005). Laperche et al. (2006b) evaluated NUE and N uptake using carbon and N sources and studied root architecture in winter wheat. They detected 32 QTL on 1A, 1B, 2B, 4B, 5A, 5B, 5D, 6A, 6B, 7A, 7B and 7D and 6 QTL for root traits. An et al. (2006) detected QTL governing N uptake during early development in wheat under low N supply. There is also considerable correlation of QTL for NUE with N metabolic pathways and enzyme activities such as GS and glutamate dehydrogenase (GDH) (Bertin and Gallais 2001). Fontaine et al. (2009) in an integrated experiment of agronomic, physiological and molecular aspects of NUE, investigated the role of GS and GDH activities and other N-related physiological traits for GY improvement in wheat. They also identified a coincidence between a QTL for GDH activity and the gene on chromosome 2B encoding GDH. In major cereal crops, the measurement of GS enzyme activities revealed co-localisations between a QTL for GS activity and a QTL for yield, suggesting the critical importance of GS activity for grain N content and yield. (Chardon et al. 2012).

Charmet et al. (2005) studied QTL in wheat and identified some significant QTL, mostly on chromosome 7A, for GY, N accumulation in grain and storage protein fraction. Laperche et al. (2007) detected some QTL for GY, PY, total N amount (shoot plus grain) and nitrogen harvest index (NHI) in wheat through studying a mapping population of 222 doubled haploid lines (DH), carried out in seven different environments at both high N and low N supply. Habash et al. (2007) reported the first study of the detection of QTL related to components of

flag leaf N metabolism during grain filling in wheat. Gallais and Hirel (2004), located fewer QTL for N-uptake than for N-utilisation efficiency under low N, whereas the reverse was true under high N. These contrasting results indicate that the plant growing under low N conditions relies on different physiological mechanisms which vary among different genotypes. Coque and Gallais (2006) analysed genome regions indicating co-localisation of QTL for NUE with QTL for NUE-related traits. At low N, they detected four such regions: one for GY and N uptake on chromosome 1A, one for GY and earliness on chromosome 2B, and two on chromosome 2D, one for GY and grain filling and the other for GY and seed setting.

In most QTL studies, the NUE concept has been accounted as the combination of two main components, NupE and NutE (Brauer and Shelp 2010). In wheat, An et al. (2006) mapped QTL for NupE by estimating the total N accumulated in the above ground parts (straw plus grain). In barley, Mickelson et al. (2003) mapped QTL for N remobilisation using the N balance method that requires monitoring the difference in flag leaf N content between anthesis and maturity. Two distinct N fluxes can be considered during the grain filling period: N remobilisation from the leaves and N uptake in roots. In maize, Coque et al. (2008) used ^{15}N-labelling to evaluate the proportion of N remobilised into kernels and the proportion of N absorbed post-silking and allocated to the grain.

Recent genetic studies on NUE, where multiple traits were studied, resulted in the detection of a large number of QTL. In wheat, Habash et al. (2007) identified 163 QTL from 21 traits, Laperche et al. (2007) studied 10 traits in seven different environments resulting in the mapping of 233 QTL, and Fontaine et al. (2009) mapped 148 QTL for seven physiological and five agronomic traits. Bogard et al. (2011) localised QTL for the three correlated traits; leaf senescence during grain filling, grain protein concentration, and GY in a doubled haploid mapping population of winter wheat in a range of multi-environment trials. Chromosomes 2A (detected as the most stable QTL for GPC), 2D and 7D were coincident for these traits, also associating with QTL for anthesis date. They proposed that the varying effect of delaying leaf

senescence on GY and GPC might depend on the availability of N particularly after anthesis and also environmental conditions. Accordingly, late leaf senescence could be a criterion for selection to improve the traits studied. They also reported one stable QTL for GPC on 2A and another one for GY on 7D, which confirmed previous detection on 7D for GY by Groos et al. (2003) in a different population. Guo et al. (2012) identified 380 QTL in a study of morphological, nutrient content and nutrient utilisation efficiency traits using a recombinant inbred line (RIL) wheat population under varying concentrations of N, P and K nutrients in hydroponic culture. Some of these regions were coincident with other results of traits studied in field conditions (Quarrie et al. 2005; Laperche et al. 2007; Fontaine et al. 2009). The responses to N level for GY and GPC were estimated in wheat using the difference and the ratio of values at the two input levels and also the slope of joint regression (Bordes et al. 2013). The authors identified significant regions for GY on almost all chromosomes except for 4B and the group 5 chromosomes, with common QTL for both GY and GPC on chromosomes 1A, 1B, 1D, 2B, 3B, 4A, 5B, 6A and 6B. They also highlighted mapped key enzymes involved in N metabolism in wheat on chromosomes 2A, 2D, 3A, 3B, 4A, 5D and 6A which co-located with previous research.

To produce an overview of the chromosome areas involved in the trait variation, a meta-analysis of the QTL approach was proposed to synthesise all the individual QTL experiments that have been carried out in different populations and using different maps. After projecting QTL on the consensus map, the meta-analysis resulted in a number of consensus chromosome regions (called meta-QTL) involved in trait variation with increased accuracy compared with the position estimated for individual QTL studies (Goffinet and Gerber 2000; Coque et al. 2008). First used by Chardon et al. (2004) to study QTL related to flowering time in maize, the meta-analysis approach is now widely performed in other crops. The later studies revealed meta-QTL for yield in rapeseed (Shi et al. 2009), oil content in soybean (Qi et al. 2011), and yield compounds in maize (Li et al. 2011). Recently, Quraishi et al. (2011) reported the first integration of the known QTL for NUE and provided an overall view of the major NUE meta-

QTL in bread wheat. In this study, a new development of the meta-analysis approach was performed by using cross-genome comparison and a synteny-based physical map. On wheat chromosome 3B, the authors mapped meta-QTL for NUE and demonstrated that a GOGAT gene conserved in three other cereal genomes; maize, rice, and sorghum is contributed to NUE in wheat via a model for the paleo-history of the NUE locus.

In the future, developments in molecular marker technology and the recording of hundreds of natural populations in crops will facilitate association mapping by offering new opportunities to discover loci statistically correlated with traits related to NUE. The association mapping method investigates the relationship between genetic markers and phenotypes in unrelated individuals by exploiting historical recombination events and genetic diversity (Ikram and Chardon 2010).

Although there have been many published NUE-QTL studies few have focussed on the Mediterranean-type environments such as in Southern Australia (Elouafi et al. 2000; Merah 2001). Therefore, there is a need for more studies that dissect the genetic variation in NUE in order to improve NUE using the integration of physiological and molecular approaches under field and controlled conditions

2.4.3.2 Genetically modified organism (GMO)

Applying modern technology such as genetic engineering is another genetic approach to understand the genetic background of NUE and find the best and quickest tools to improve NUE, although the lack of known genes directly associated with NUE makes this difficult (Hao et al. 2011). Hao et al. (2011) identified significant responses to low N stress using differential transcript abundance and gene expression analysis by Digital Gene Expression (DGE) profiling in soybean. Man et al. (2005) demonstrated that the enhanced expression of GS in transgenic poplar, characterised by the ectopic expression of pine cytosolic GS, resulted in improvement of N assimilation and enhanced growth. In wheat, Habash et al. (2001) analysed transgenic lines with increased GS1 activity in leaves indicating that N accumulation, mostly in grain and roots, were enhanced. They also showed the possibility of

manipulation of GS in order to improve N use in wheat. There are several other studies that used a transgenic approach to improve NUE. Other engineered genetic modified plants could overexpression of alanine aminotransferase (AlaAT) showing enhanced biomass and GY at low N in both field and controlled conditions (Good et al. 2007). Another AlaAT NUE technology to improve NUE was done in rice. Shrawat et al. (2008) developed genetically N-efficient rice (*Oryza sativa* L.) by introducing a barley *AlaAT* cDNA driven by a rice tissue-specific promoter (*OsAnt1*). The transgenic plants had increased biomass, GY and also high N content resulted in improved NUE.

2.5 Objectives

The main objectives of this thesis were to:

1) Examine the genetic variation for NUE that is present between Southern Australian wheat varieties and investigate the cause of the variation in NUE

2) Identify QTL controlling NUE and NUE-related traits in a Southern Australian bi-parental mapping population

3) Investigate the impact of field NUE QTL on N flux under controlled environmental conditions.

Chapter 3

Statement of Authorship

Title of Paper: Evaluation of Australian wheat genotypes for response to variable nitrogen application

Publication Status: Submitted for Publication

Publication Details: Saba Mahjourimajd, Haydn Kuchel, Peter Langridge, Mamoru Okamoto, 2014, Plant Soil

Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Saba Mahjourimajd Performed analysis on all samples, interpreted data, wrote manuscript and acted as corresponding author

Signature **Date** 17/12/14

Dr. Haydn Kuchel Supervised development of work, helped in data interpretation and manuscript evaluation

Signature **Date** 15/12/14

Prof. Peter Langridge Supervised development of work, helped in data interpretation and manuscript evaluation

Signature **Date** 15/12/2014

Dr. Mamoru Okamoto Supervised development of work, helped in data interpretation and manuscript evaluation

Signature **Date** 15/12/2014

Evaluation of Australian wheat genotypes for response to variable nitrogen application

Saba Mahjourimajd[1], Haydn Kuchel[2], Peter Langridge[1], Mamoru Okamoto[1]

[1]Australian Centre for Plant Functional Genomics (ACPFG), University of Adelaide, PMB1, Glen Osmond, SA 5064, Australia

[2]Australian Grain Technologies, PMB1, Glen Osmond, SA 5064, Australia

Abstract

Aims: The key aim was to assess the genetic variation for nitrogen (N) response and stability in spring wheat germplasm to determine the scope for improvement of nitrogen use efficiency (NUE) under water-limited and low-yielding conditions. A further aim was to evaluate NUE stability and NUE-protein yield (PY) as suitable NUE-related traits for selection.

Methods: The traits measured included grain yield (GY, kg ha^{-1}) and NUE (kg GY kg^{-1} N) under varying N applications at all sites, and NUE for protein yield (NUE-PY), harvest index (HI) and plant height (H) at some sites. In addition, two of the trials used two seeding rates to provide an assessment of the impact of plant density on NUE.

Result: Genetic variation was significant for all traits studied. Grain yield was affected by both genotype (G) and N rate and the interaction between the two. Interestingly, harvest index and height showed no direct response to varying N applications. However, there was a significant G effect and N response (G×N interaction).

Conclusions: Increasing N inputs led to variable responses for GY at different sites. Importantly, the genetic variation in N response should enable plant breeders to select consistently high N responsive wheat genotypes to improve NUE.

Keywords Wheat; Nitrogen response; Nitrogen use efficiency; Grain yield; Protein yield

Authors:

Saba Mahjourimajd (saba.mahjourimajd@adelaide.edu.au)- Haydn Kuchel (haydn.kuchel@ausgraintech.com)- Peter Langridge (peter.langridge@adelaide.edu.au) - Mamoru Okamoto (mamoru.okamoto@acpfg.com.au)

Corresponding author:

Saba Mahjourimajd

e-mail: saba.mahjourimajd@adelaide.edu.au

Office phone: +61 8 83131498

Fax No: +61 8 8313 7102

Abbreviations

Nitrogen	N
Nitrogen use efficiency	NUE
Genotype	G
Grain yield	GY
Seeding rate	SR
Protein yield	PY
Plant height	H
Harvest index	HI

Introduction

The global rate of consumption for N fertiliser is higher than for any other nutrient (62% of all fertiliser application is N) (FAO 2011). However, studies have demonstrated that cereals, including wheat, cannot effectively utilise the supplied N and it is estimated that only 40-60% of N supplied is absorbed by crops (Craswell and Godwin 1984; Hodge et al. 2000; Sylvester-Bradley and Kindred 2009). This low uptake of N can affect NUE and lead to high production costs, loss of N from the soil by leaching, contamination of surface and underground water (Mizuta et al. 2004), and gaseous emissions such as nitrous oxide, a major greenhouse gas (Harrison and Webb 2001). In addition, the poor use of supplied N may lead to insufficient N availability for plants at times of peak demand with consequent yield reduction. Genotypes show different behaviour with different levels of available N across sites and growing seasons (Le Gouis and Pluchard 1996; Gallais and Coque 2005; An et al. 2006). The two main components of NUE, N uptake efficiency and N utilisation efficiency, should both be taken into account to optimise NUE in plants. Both components are controlled by genetic and environmental factors indicating varying performances across genotypes (Gallais and Coque 2005; Laperche et al. 2006; Coque et al. 2008).

A prime challenge for plant breeders is screening and selection of genotypes for consistent N response and high NUE in order to reduce N losses and maximise yield and other desirable traits. To meet this challenge, we need a detailed understanding of available genetic variation in N response, using field and controlled environment approaches to assess the responsiveness of genotypes to supplied N, and dissection of N metabolic pathways. However, NUE and N response are complex traits which show inconsistent trends across years and sites (Hirel et al. 2001; Chen et al. 2004; Brennan et al. 2014). Accordingly, the integration of agronomic, physiological and molecular data will be important for selection of the best genotypes with high NUE in specific environments (Hirel et al. 2007; Pathak et al. 2008; Sylvester-Bradley and Kindred 2009). Several researchers have considered the yield response of genotypes under varying growth conditions. Since G×E seriously complicates genetic improvement for GY in wheat (Cooper et al. 1996), Podlich et al. (1999) proposed a selection strategy to accommodate G×E via computer simulation in multi-environment trials.

The goal of NUE improvement is to increase grain production via either direct selection for GY or indirect selection for yield components. For example, there is a significant relationship between yield and biomass and thousand kernel weight with application of N fertiliser (Serrano et al. 2000; Groos et al. 2003). Kanampiu et al. (1997) reported that genotypes with high HI (grain produced divided by the total dry biomass) demonstrated a higher NUE and potentially increased GY (Raun and Johnson 1999). Nitrogen assimilation,

particularly close to anthesis and during remobilisation into the grain, affects the duration of grain filling and therefore yield (Barbottin et al. 2005), and highlights the need for sufficient N supply throughout crop growth. One of the main components of NUE is expected to be HI (Le Gouis et al. 2000).

Nitrogen and water deficit can affect GY differently but in most wheat production areas the two factors are likely to be linked. Co-occurrence of low N and low water availabilities has been reported for the Mediterranean-type environment of South Australia (Angus and Van Herwaarden 2001; Sadras et al. 2012). Relatively little is known about the interaction of water stress and N availability or the effects of the combination of these two factors on yield components. The aims of this study was to study genetic variation in modern Australian genotypes for N response and NUE stability to screen and select for the genotypes with consistent and high NUE under conditions where water is limited and yields are low.

Materials and Methods

Field experiments

Five NUE field trials were conducted in a split-plot design with three replicates at varying rates of N application in different sites of South Australia in 2010 and 2011. Nitrogen treatments were applied to the main plots with the genotypes grown in sub-plots. Nitrogen rates varied between 18 and 87 kg N ha^{-1} at either 3 or 4 levels. At two sites, two different seeding rates were also used (Table 1). Urea N fertiliser was applied once at planting time. The geographic and climate information, soil conditions, experimental design and average GY at each site are also presented in Table 1. A set of 24 Australian genotypes of spring wheat (*Triticum aestivum* L.) was cultivated (Table 2). The genotypes were composed of modern elite lines, and parents of mapping populations. Weed and insect control treatments followed standard practice for the region. Soil samples were taken from the field before planting, and analysed for a range of characters including N levels by CSPB (Bibra Lake, WA, Australia) (Table 1).

Data collection and calculations

Grain yield (GY, kg ha^{-1}), at harvest time where moisture content was around 15%, and NUE (GY per unit of N supplied, kg GY kg^{-1} N) were measured at all sites. Residual nitrogen was low at all sites and was not included in the calculations of NUE (Moll et al. 1982). Calculated NUE stability (NUE at high N – NUE at low N, kg ha^{-1}) reflected the responsiveness of NUE at high N supply. The genotypes were ranked for NUE stability on a scale of 1 to 8 (1=high NUE stability; 8=low NUE stability). The efficiency of N for protein yield (NUE-PY; grain protein content per unit of N supplied, kg PY kg^{-1} N) was determined at two sites, CUM 11 and ROS 11. At physiological maturity, H (cm) at spike neck was measured at ROS 11.

Above-ground HI (the proportion of grain dry matter to total shoot dry matter, %) was determined at physiological maturity, from a 50 cm interval of the two central rows in each plot at ROS 11.

Statistical analysis

All data were used in spatial analysis to estimate the predicted means of the traits of interest using the REML directive in GenStat (VSN international, Version 15) (Payne 2009) (Tables 3 and 4). The predicted means were used to compare genotypes for the traits of interest across different sites and N treatments. The phenotypic correlation coefficient, according to the predicted means, was measured for the traits interest (Table 5).

Results

Significant genetic variation existed for GY and consequently NUE at each of the trial sites (Table 3). In this study, the average GY was highest at MIN 10 and lowest at PIN 10 (Table 1). The effect of N treatment was significant for GY at all sites. A genotype-by-N treatment interaction (G×N) for GY was significant only at ROS 11 ($P < 0.05$). For NUE, G and N application level had significant effects ($P < 0.001$) in all sites although the effects were not consistent. Genotypes responded differently to N fertilisation which showed a significant interaction of G×N for NUE at all sites except TUC 10. An interaction of seeding rate (SR) with N treatment for GY and NUE was significant at CUM 11 and ROS 11. The ratio of grain protein content to N supplied (NUE-PY), at CUM 11 and ROS 11 was influenced by G, N treatment and SR, but a G×N interaction was only observed at CUM 11. Nitrogen use efficiency for PY (NUE-PY) was highly correlated with NUE at CUM 11 (r=0.79). At ROS 11, where genotypes were scored for H, the effects of G, SR, G×N, G×SR and N×SR interaction were significant for H, while the effect of N was not significant (Table 4). There was genotypic variation for HI at ROS 11 (Table 4). Although N application did not have a significant effect on HI, there was a significant effect of G×N. There was no significant interaction of G×N×SR for any of the traits measured in this study (Tables 3 and 4). Although the average correlation for variety performance for GY between sites was relatively large (0.52), the correlation between sites for N response was smaller (Table 5). The results in this study showed that genotypes with higher HI tended to also show higher NUE (Supplementary Table 1). The R^2 for the relationship between HI and NUE was 0.46 (Fig. 1). However, some varieties showed consistent responses to N across sites. As expected, increased supply of N resulted in higher GY, but reduced NUE (Fig. 2). Between sites, MIN 10 showed the highest NUE at the high rate of N application, but it was still much lower than the NUE observed for the low N treatment. With increasing N application, NUE differed significantly in most sites

except at TUC 10 (Fig. 2). In order to investigate stability of NUE and N responsiveness, several genotypes were compared across the four sites (MIN 10, PIN 10, CUM 11, and ROS 11). Fig. 3 shows the ranked responsiveness of genotypes for NUE at trial sites with a significant G×N effect. Overall, Mace and RAC1569 ranked highly and were stable for NUE across sites and N treatments, while Frame, Kord CL Plus and Catalina ranked poorly.

Discussion

In this study, we observed low GY at low N supply across sites, consistent with the results of Abe et al. (2013), Cormier et al. (2013) and Pang et al. (2013). However, in contrast to previous studies, we explored genetic variation for NUE under low-yielding conditions where productivity is severely limited by moisture stress. Under these production conditions, nitrogen is often applied at or near sowing and the ability of the crop to manage N uptake and use N during crop establishment and early growth is critical. Excessive early vigour and biomass production in response to the available N, can be a liability late in the season when water is severely limited. The varieties were ranked for NUE across trials sites to reveal lines showing stable performance across environments. In line with previous reports, (Bertin and Gallais 2001; An et al. 2006; Asplund et al. 2014) the present study demonstrated that there is significant variation for NUE within current wheat genotypes. Abe et al (2013) also demonstrated significant genetic variation for GY and measured NUE component traits among maize hybrids at increasing rates of N application. In another study, the genetic progress for NUE in winter wheat (*Triticum aestivum* L.) was assessed in two hundred and twenty-five elite European varieties at four sites under two levels of N application. Significant effects of genotype for GY and NUE in all sites and G×N interactions at some sites, were in line with the previous studies (Cormier et al. 2013). Peng et al (2013) determined the critical soil mineral N concentration to achieve optimum GY in maize in a three-year field trial at three N treatments. Their findings concur with the results from our study where we demonstrated significantly higher NUE at high levels of N treatment relative to the low N treatments. The genetic basis for variation in NUE has been studied in NUE-Quantitative Trait Loci (QTL) studies. For example, Bertin and Gallais (2001) in a study of genetic variation for NUE in a set of maize recombinant inbred lines, detected significant QTL for GY at high N and low levels of N. In addition, Asplund et al (2014) evaluated a new concept for assessment of NUE in six spring wheat varieties under field and controlled conditions. However, the impact of environment (the interaction of climate, soil, water availability and other factors) and G×E on NUE and N responsiveness confirms that achieving genetic gains for NUE will be challenging (Ortiz-Monasterio et al. 1997; Hirel et al. 2007). It is likely that

variation in the timing and amount of rainfall, as well as other abiotic stresses such as hot days (Table 1), influenced the value of N application at each site, and the relative response of each genotype to N supply in this study. For instance, at MIN 10, GY was, on average, higher than other sites due to higher rainfall and fewer hot days during the growth season relative to most sites. However, GY was relatively high at CUM 11 with lower rainfall and more days with high temperature. The soil conditions and N components could have affected the final productivity at this site. Further, the lowest GY at PIN 10, could be related to the sub-optimal rainfall and high number of hot days with poor soil N (Table 1). The results showed the effect of the interaction between environmental factors and N supplied; particularly the effect of both water and N availability on the final production at the different sites. Interestingly, SR was found to interact with N supply and G, although there was no G×N×SR interaction. This result needs to be confirmed, as it was only tested at two sites with a limited set of genotypes, but if correct it helps to simplify the challenges of improving NUE in wheat. Geleta et al. (2002) demonstrated that different genotypes did not necessarily show the same response for both GY and SR. GY also varies at different N levels. Extensive interaction between SR and N would hamper improvement for GY under variable N. Therefore, the fewer interacting factors the more manageable the task of improvement for complex traits such as NUE.

Kanampiu et al. (1997) described that low N loss was associated with high HI and low forage yields in winter wheat which resulted in high NUE. Similarly, genotypes with high HI showed improved NUE at ROS 11 (Fig. 1 and Supplementary Table 1). With respect to H, taller genotypes at ROS 11 showed, on average, lower NUE. This observation is also in agreement with other studies. For instance, Guarda et al. (2004) reported that reduced H led to an increase in HI, and proposed that this was due, at least partially, to more efficient partitioning of photosynthates to the developing spike. Water deficit and high temperatures occur later in the season during flowering and grain filling in the production environments of southern Australia. In the region used for this study, crops are sown after autumn rainfall when good soil moisture is available for crop establishment and early vegetative growth. Varieties able to take-up N during early growth but restrict tillering and vegetative growth are less susceptible to the late season drought but need to efficiently remobilise the N during grain filling (Liao et al. 2004). As expected, the protein content of genotypes responded differently to N levels for the two sites where this was measured, CUM 11 and ROS 11, indicating the interaction of N and environmental factors. There was a large correlation between NUE and NUE-PY suggesting that NUE-PY may be considered as a component of NUE in plant breeding (r= 0.79). The effects of NUE on both grain quantity and quality characteristics need to be considered (Peterson et al. 1992; Uribelarrea et al. 2009).

Some genotypes which responded strongly to N fertilisation, were low-yielding and had low NUE at low N supply (for example, Kord CL Plus at PIN 10, Axe at CUM 11, Grenade at ROS 11). The opposite was also true, where some varieties such as Corack at CUM 11, Excalibur at MIN 10, Wyalkatchem at PIN 10, RAC875 at ROS 11 and TUC 10 and Frame at TUC 10 showing high GY at low N supply and no strong response to increased N application**.** These results confirm the G×E effects on the performance of different genotypes and supports a previous study in oilseed rape (Ulas et al. 2013) which suggested that efficiency and responsiveness may need to be considered independently. The negative association between efficiency and responsiveness may relate to the issue raised earlier about the ability of plants to manage early growth and N uptake to limit stress susceptibility late in the season. The results could reflect two approaches to deal with this problem. Genotypes such as Kord CL, Axe and Grenade, may use the available N to build biomass but are then limited for N late in the season which results in low yields and low NUE. The opposite group may be better able to match biomass production to N supply, allowing them to restrict early growth to ensure adequate N will be available during flowering and grain filling. These lines are able to show a consistent response to N across multiple environments. To improve NUE both efficiency and responsiveness to NUE should be considered making this second group particularly interesting. The present and previous studies emphasised the need for a clear definition of N responsiveness and NUE, especially for breeding objectives. The objectives could include selection of genotypes with the capability to maintain high yield under low N input, or developing genotypes with high N responsiveness and high NUE at high N supply.

In an effort to identify genotypes that have high NUE at both low and high N supply, the consistency or stability of the N response is important. Breeders will aim to identify genotypes which show high and consistent NUE across N application levels and sites. Although NUE stability showed smaller genetic correlation across sites than GY (Table 5), some genotypes were identified that ranked highly for NUE stability indicating consistent response for NUE, at all sites, while other genotypes showed large variation (inconsistent or low NUE stability) between sites. In sites where a significant G×N effect was observed for NUE, Mace and RAC1569 showed stable and high NUE (Fig. 3), suggesting that these genotypes could be exploited by breeders to improve NUE. In contrast, the NUE stability of Frame varied between sites, and Kord CL Plus had a low NUE stability at most sites.

In conclusion, we identified genetic variation for NUE-related traits among the selected modern Australian genotypes grown in low-yielding environments. We were able to select and rank genotypes for NUE stability or consistency of the N response suggesting the potential use of this trait for G×N evaluation even across different yielding environments. The

rankings for NUE stability and NUE-PY can be applied to selection for generating new mapping populations to dissect the genetic basis of the contrasting performance for NUE. Ultimately, the knowledge can be passed to the wheat breeding programs to develop genotypes with improved NUE.

Acknowledgements

The authors would like to thank the staff at Australian Grain Technologies (AGT) for planting and managing the NUE experiments. Also thanks to Dr. Dion Bennett for his assistance and suggestions during the field work. This work was supported through funding from the Grains Research and Development Corporation, the Australian Research Council, the University of Adelaide and the Premier's Research and Industry Fund provided by the South Australian Government Department of Further Education, Employment, Science and Technology. Thanks also to Dr. Julie Hayes for critical reading and helpful comments on the manuscript.

References

Abe A, Menkir A, Moose SP, Adetimirin VO, Olaniyan AB (2013) Genetic variation for nitrogen-use efficiency among selected tropical maize hybrids differing in grain yield potential. J Crop Improv 27:31-52

An D, Su J, Liu Q, Zhu Y, Tong Y, Li J, Jing R, Li B, Li Z (2006) Mapping QTLs for nitrogen uptake in relation to the early growth of wheat (*Triticum aestivum* L.). Plant Soil 284:73-84

Angus J, Van Herwaarden A (2001) Increasing water use and water use efficiency in dryland wheat. Agron J 93:290-298

Asplund L, Bergkvist G, Weih M (2014) Proof of concept: nitrogen use efficiency of contrasting spring wheat varieties grown in greenhouse and field. Plant Soil 374:829-842

Barbottin A, Lecomte C, Bouchard C, Jeuffroy M-H (2005) Nitrogen remobilization during grain filling in wheat. Crop Sci 45:1141-1150

Bertin P, Gallais A (2001) Genetic variation for nitrogen use efficiency in a set of recombinant inbred lines II - QTL detection and coincidences. Maydica 46:53-68

Brennan J, Hackett R, McCabe T, Grant J, Fortune R, Forristal P (2014) The effect of tillage system and residue management on grain yield and nitrogen use efficiency in winter wheat in a cool Atlantic climate. Eur J Agron 54:61-69

Chen C, Westcott M, Neill K, Wichman D, Knox M (2004) Row configuration and nitrogen application for barley–pea intercropping in Montana. Agron J 96:1730-1738

Cooper M, DeLacy I, Basford K (1996) Relationships among analytical methods used to analyse genotypic adaptation in multi-environment trials. Plant adaptation and crop improvement, 193-224. CAB Int., Wallingford, UK

Coque M, Martin A, Veyrieras J, Hirel B, Gallais A (2008) Genetic variation for N-remobilization and postsilking N-uptake in a set of maize recombinant inbred lines. 3. QTL detection and coincidences. Theor Appl Genet 117:729-747

Cormier F, Faure S, Dubreuil P, Heumez E, Beauchêne K, Lafarge S, Praud S, Le Gouis J (2013) A multi-environmental study of recent breeding progress on nitrogen use efficiency in wheat (*Triticum aestivum* L.). Theor Appl Genet 126:3035-3048

Craswell E, Godwin D (1984) The efficiency of nitrogen fertilizers applied to cereals grown in different climates. Adv Nutr 1:1-55

FAO (2011) Current world fertilizer trends and outlook to 2015. ftp://ftp.fao.org/ag/agp/docs/cwfto15.pdf.

Gallais A, Coque M (2005) Genetic variation and selection for nitrogen use efficiency in maize: a synthesis. Maydica 50:531-547

Geleta B, Atak M, Baenziger P, Nelson L, Baltenesperger D, Eskridge K, Shipman M, Shelton D (2002) Seeding rate and genotype effect on agronomic performance and end-use quality of winter wheat. Crop Sci 42:827-832

Groos C, Robert N, Bervas E, Charmet G (2003) Genetic analysis of grain protein-content, grain yield and thousand-kernel weight in bread wheat. Theor Appl Genet 106:1032-1040

Guarda G, Padovan S, Delogu G (2004) Grain yield, nitrogen-use efficiency and baking quality of old and modern Italian bread-wheat cultivars grown at different nitrogen levels. Eur J Agron 21:181-192
Harrison R, Webb J (2001) A review of the effect of N fertilizer type on gaseous emissions. Adv Agron 73:65-108
Hirel B, Bertin P, Quilleré I, Bourdoncle W, Attagnant C, Dellay C, Gouy A, Cadiou S, Retailliau C, Falque M (2001) Towards a better understanding of the genetic and physiological basis for nitrogen use efficiency in maize. Plant Physiol 125:1258-1270
Hirel B, Le Gouis J, Ney B, Gallais A (2007) The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. J Exp Bot 58:2369-2387
Hodge A, Robinson D, Fitter A (2000) Are microorganisms more effective than plants at competing for nitrogen? Trends Plant Sci 5:304-308
Kanampiu FK, Raun WR, Johnson GV (1997) Effect of nitrogen rate on plant nitrogen loss in winter wheat varieties. J Plant Nutr 20:389-404
Laperche A, Devienne-Barret F, Maury O, Le Gouis J, Ney B (2006) A simplified conceptual model of carbon/nitrogen functioning for QTL analysis of winter wheat adaptation to nitrogen deficiency. Theor Appl Genet 113:1131-1146
Le Gouis J, Béghin D, Heumez E, Pluchard P (2000) Genetic differences for nitrogen uptake and nitrogen utilisation efficiencies in winter wheat. Eur J Agron 12:163-173
Le Gouis J, Pluchard P (1996) Genetic variation for nitrogen use efficiency in winter wheat (*Triticum aestivum* L.). Euphytica 92:221-224
Liao MT, Fillery IRP, Palta JA (2004) Early vigorous growth is a major factor influencing nitrogen uptake in wheat Funct Plant Biol 31:121-129
Mizuta K, Matsumoto T, Hatate Y, Nishihara K, Nakanishi T (2004) Removal of nitrate-nitrogen from drinking water using bamboo powder charcoal. Bioresour Technol 95:255-257
Moll RH, Kamprath EJ, Jackson WA (1982) Analysis and interpretation of factors which contribute to efficiency of nitrogen-utilization. Agron J 74:562-564
Ortiz-Monasterio R, Sayre K, Rajaram S, McMahon M (1997) Genetic progress in wheat yield and nitrogen use efficiency under four nitrogen rates. Crop Sci 37:898-904
Pang J, Palta JA, Rebetzke GJ, Milroy SP (2013) Wheat genotypes with high early vigour accumulate more nitrogen and have higher photosynthetic nitrogen use efficiency during early growth. Funct Plant Biol 41:215-222
Pathak RR, Ahmad A, Lochab S, Raghuram N (2008) Molecular physiology of plant nitrogen use efficiency and biotechnological options for its enhancement. Curr Sci 94:1394-1403
Payne RW (2009) GenStat, Wiley Interdisciplinary Reviews. Comput Stat 1:255-258
Peng Y, Yu P, Li X, Li C (2013) Determination of the critical soil mineral nitrogen concentration for maximizing maize grain yield. Plant soil 372:41-51
Peterson C, Graybosch R, Baenziger P, Grombacher A (1992) Genotype and environment effects on quality characteristics of hard red winter wheat. Crop Sci 32:98-103
Podlich D, Cooper M, Basford K, Geiger H (1999) Computer simulation of a selection strategy to accommodate genotype environment interactions in a wheat recurrent selection programme. Plant Breeding 118:17-28
Raun WR, Johnson GV (1999) Improving nitrogen use efficiency for cereal production. Agron J 91:357-363
Sadras VO, Lawson C, Hooper P, McDonald GK (2012) Contribution of summer rainfall and nitrogen to the yield and water use efficiency of wheat in Mediterranean-type environments of South Australia. Eur J Agron 36:41-54
Serrano L, Filella I, Penuelas J (2000) Remote sensing of biomass and yield of winter wheat under different nitrogen supplies. Crop Sci 40:723-731
Sylvester-Bradley R, Kindred DR (2009) Analysing nitrogen responses of cereals to prioritize routes to the improvement of nitrogen use efficiency. J Exp Bot 60:1939-1951
Ulas A, Behrens T, Wiesler F, Horst WJ (2013) Does genotypic variation in nitrogen remobilisation efficiency contribute to nitrogen efficiency of winter oilseed-rape cultivars (*Brassica napus* L.)? Plant soil 371:463-471
Uribelarrea M, Crafts-Brandner SJ, Below FE (2009) Physiological N response of field-grown maize hybrids (*Zea mays* L.) with divergent yield potential and grain protein concentration. Plant soil 316:151-160

Table 1 The location, climatic and basic soil characteristics, growing conditions and average grain yield (GY, kg ha^{-1}) of five trial sites in South Australia selected for nitrogen use efficiency field trials in 2010- 2011

Site	Year	Abbreviation	Lat[a] (°S)	Lon[b] (°E)	Elv[c] (m)	Total rain[d] (mm)	Hot day[e] (d)	Soil Texture[f]	pH level ($CaCl_2$)	pH level (H_2O)	NH_4^+ nitrogen (mg kg^{-1})	NO_3^- nitrogen (mg kg^{-1})	Nitrogen fertiliser levels (kg ha^{-1})	Seeding rate level (seed m^{-2})	Average GY (kg ha^{-1})
MINTARO	2010	MIN 10	33.9	138.7	418	522	14	Heavy clay	7	7.8	11	8	18, 50, 64, 87	200	5243
PINNAROO	2010	PIN 10	35.2	140.9	101	373	20	Loamy sand	6.6	7.5	8	9	18, 50, 64, 87	200	2476
TUCKEY	2010	TUC 10	33.7	136.5	193	348	15	Loamy sand	7.2	7.8	2	8	18, 50, 64, 87	200	3107
CUMMINS	2011	CUM 11	34.2	135.7	65	242	22	Loamy sand	6.3	6.8	3	26	18, 41, 87	100, 200	4261
ROSEWORTHY	2011	ROS 11	34.5	138.7	103	251	30	Heavy clay	6.9	7.5	2	18	18, 41, 87	100, 200	3598

[a] Latitude (°S)
[b] Longitude (°E)
[c] Elevation above sea level (Elv, m)
[d] Total rainfall during growth season
[e] Number of growth season hot days with temperature higher than 30 °C
[f] Soil characteristics at top 10 cm depth of soil before fertilisation

Table 2 Spring wheat genotypes studied at five nitrogen use efficiency field trial site locations, 2010- 2011

Genotype[a]	Abbreviation
AGT-KATANA	KAT
AXE	AXE
CATALINA	CAT
CORACK	CORA
CORRELL	COR
DERRIMUT	DER
DRYSDALE	DRY
ELMORE CL PLUS	ELM
ESPADA	ESP
ESTOC	EST
EXCALIBUR	EXC
FRAME	FRA
GLADIUS	GLA
GRENADE CL PLUS	GRE
JANZ	JAN
JUSTICA CL PLUS	JUS
KORD CL PLUS	KOR
KUKRI	KUK
MACE	MAC
RAC0875	R875
RAC1569	R1569
SABEL CL PLUS	SAB
SCOUT	SCO
WAGT 104	WAG
WYALKATCHEM	WYA
YITPI	YIT
YOUNG	YOU

[a] Exceptions for genotypes: ELM, GRE and SCO were not included at MIN 10, PIN 10 and TUC 10, likewisely, DER, DRY and WAG at CUM 11 and ROS 1

Table 3 The significance (*P* - value) of genotype (G), N treatment (N), seeding rate (SR) and their interactions on agronomic traits, grain yield (GY, kg ha^{-1}), nitrogen use efficiency (NUE, kg GY kg^{-1} N) and nitrogen use efficiency for protein yield (NUE-PY, kg PY kg^{-1} N) in nitrogen use efficiency field trials at five sites in South Australia, 2010- 2011

Traits	Sites	Factors						
		G	N	SR	G × N	G × SR	N × SR	G × N× SR
GY	MIN 10	<0.001	<0.001	-	0.123	-	-	-
	PIN 10	<0.001	<0.001	-	0.479	-	-	-
	TUC 10	<0.001	<0.001	-	0.672	-	-	-
	CUM 11	<0.001	<0.001	<0.001	0.128	0.433	0.002	0.950
	ROS 11	<0.001	<0.001	<0.001	0.003	0.013	0.025	0.652
NUE	MIN 10	<0.001	<0.001	-	<0.001	-	-	-
	PIN 10	<0.001	<0.001	-	<0.001	-	-	-
	TUC 10	<0.001	<0.001	-	0.250	-	-	-
	CUM 11	<0.001	<0.001	<0.001	<0.001	0.745	<0.001	1.000
	ROS 11	<0.001	<0.001	<0.001	0.018	0.258	<0.001	0.661
NUE-PY	CUM 11	<0.001	<0.001	<0.001	0.002	0.609	0.008	0.999
	ROS 11	<0.001	<0.001	<0.001	0.464	0.233	0.058	0.614

Not significant, n.s at $P > 0.05$; 5% significant at $P = 0.05$; 1% significant at $P = 0.01$

Table 4 The significance (*P* - value) of genotype (G), N treatment (N), seeding rate (SR) and their interactions on agronomic traits, plant height (H, cm) and harvest index (HI, %) measured in nitrogen use efficiency field trials at ROS 11

Factors	ROS 11	
	H (cm)	HI (%)
G	<0.001	<0.001
N	0.588	0.515
SR	<0.001	0.458
G × N	<0.001	0.002
G × SR	0.003	0.771
N × SR	0.001	0.533
G × N × SR	0.229	0.967

Not significant, n.s at $P > 0.05$; 5% significant at $P = 0.05$; 1% significant at $P = 0.01$

Table 5 Correlation between sites for grain yield (GY, kg ha^{-1}), nitrogen use efficiency (NUE, kg GY kg^{-1} N) and nitrogen use efficiency (NUE) stability between sites with the significance of G×N for NUE across nitrogen treatments. Each trial was run using a split plot design with three replicates. The GY data was corrected using spatial analysis

Traits	Sites	MIN 10	PIN 10	TUC 10	CUM 11
GY	PIN 10	0.67			
	TUC 10	0.37	0.38		
	CUM 11	0.73	0.56	0.55	
	ROS 11	0.70	0.63	0.15	0.46
NUE	PIN 10	0.61			
	CUM 11	0.54	0.47	-	
	ROS 11	0.58	0.54	-	0.19
NUE Stability	PIN 10	0.41			
	CUM 11	0.23	0.17	-	
	ROS 11	0.40	0.47	-	-0.02

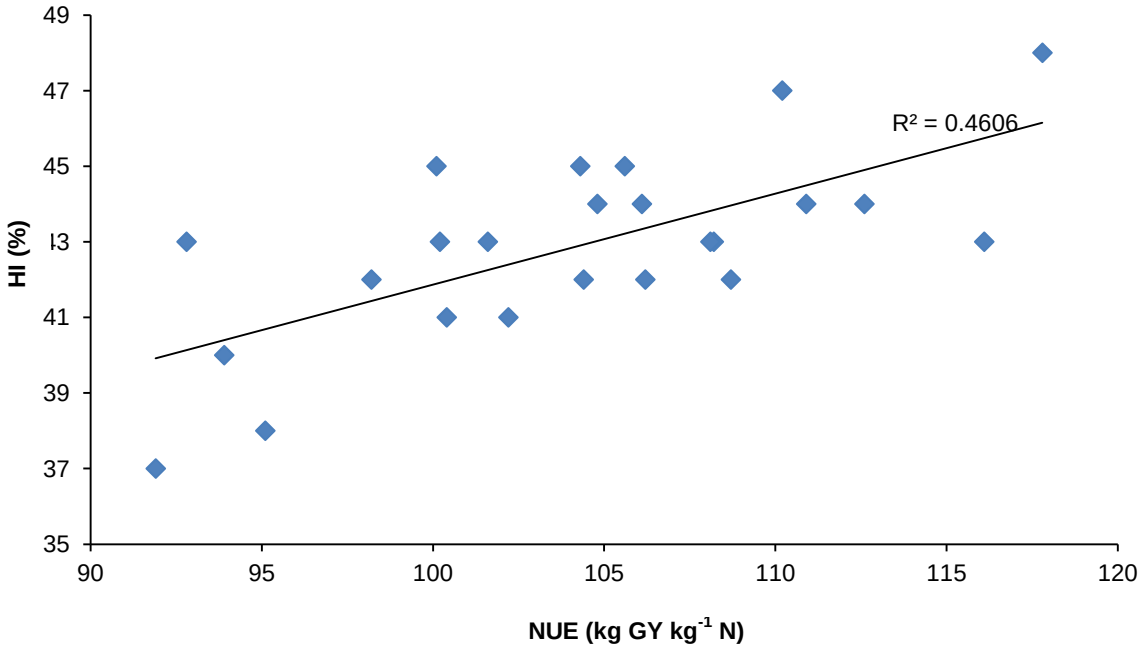


Fig. 1 The comparison of harvest index (HI, %) and nitrogen use efficiency (NUE, kg GY kg^{-1} N) values of wheat genotypes at ROS 11

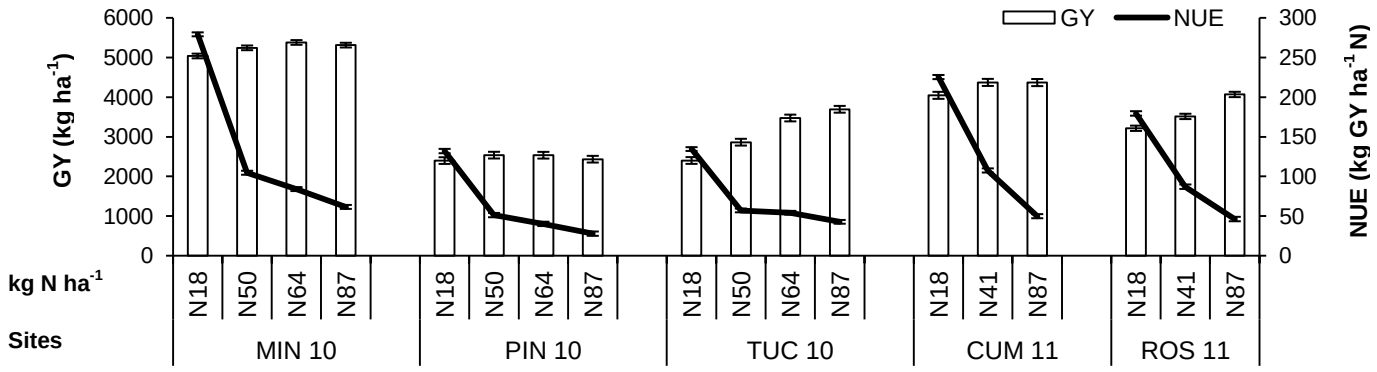


Fig. 2 The average performance of 24 wheat genotypes for grain yield (GY, kg ha^{-1}) and nitrogen use efficiency (NUE, kg GY kg^{-1} N) at varied levels of N fertilisation in five sites of South Australia, 2010- 2011. The vertical error bars represent the standard errors of the predicted means after spatial analysis

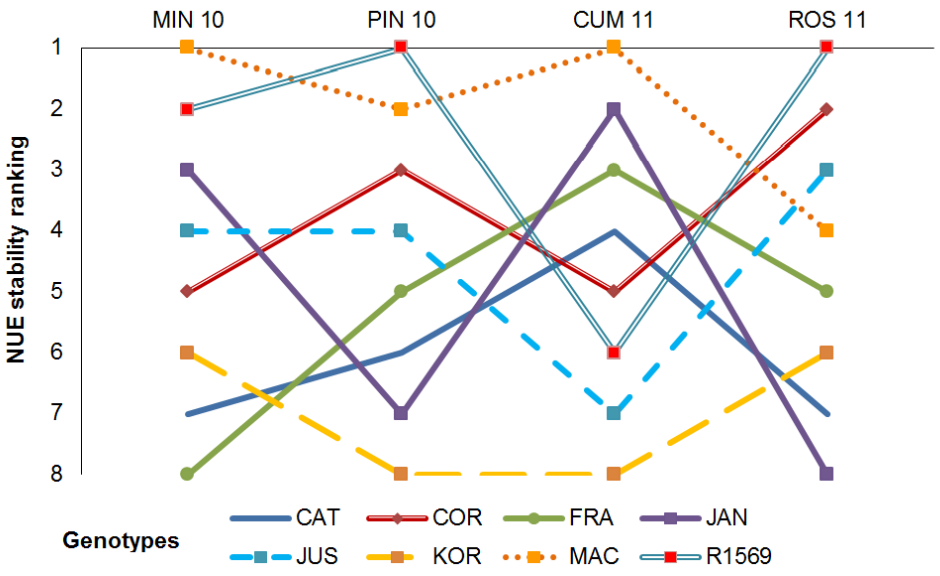


Fig. 3 Nitrogen use efficiency (NUE) stability rankings (1= high NUE stability; 8=low NUE stability) of selected wheat genotypes at sites where there was significant G×N interaction for NUE

Supplementary materials Chapter 3

Table 1 The comparison of harvest index (HI, (%) and nitrogen use efficiency (NUE, kg GY kg^{-1} N) values of wheat genotypes at ROS 11

Genotypes	HI	NUE
R1569	48	117.8
SCO	43	116.1
MAC	44	112.6
AXE	44	110.9
CORA	47	110.2
COR	42	108.7
KOR	43	108.2
R875	43	108.1
EST	42	106.2
ELM	44	106.1
YOU	45	105.6
GLA	44	104.8
JUS	42	104.4
KAT	45	104.3
ESP	41	102.2
WYA	43	101.6
KUK	41	100.4
EXC	43	100.2
YIT	45	100.1
SAB	42	98.2
GRE	38	95.1
JAN	40	93.9
CAT	43	92.8
FRA	37	91.9

Chapter 4

Statement of Authorship

Title of Paper: Genetic basis for variation in grain yield under different nitrogen amounts in wheat

Publication Status: Publication Style

Publication Details: Saba Mahjourimajd, Julian Taylor, Zed Rengel, Hossein Khabaz-Saberi, Haydn Kuchel, Mamoru Okamoto, Peter Langridge, 2014, Theoretical and Applied Genetics

Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Saba Mahjourimajd Performed analysis on all samples, interpreted data and wrote manuscript

Signature **Date** 17/12/14

Dr. Julian Taylor Analysed the data, contributed to the ideas and manuscript

Signature **Date** 10/12/2014

Prof. Zed Rengel Provided research data, contributed to research ideas and design

Signature **Date 04 Dec 2014**

Dr. Hossein Khabaz-Saberi Provision of phenotyping data of wheat DH for two sites in Western Australia

Signature **Date 09/12/2014**

Dr. Haydn Kuchel Supervised development of work, helped in data interpretation and manuscript evaluation

Signature **Date** 15/12/14

Dr. Mamoru Okamoto Supervised development of work, helped in data interpretation and manuscript evaluation

Signature **Date** 15/12/2014

Prof. Peter Langridge Supervised development of work, helped in data interpretation and manuscript evaluation

Signature **Date** 15/12/2014

Genetic basis for variation in wheat grain yield in response to varying nitrogen application

Saba Mahjourimajd[1], Julian Taylor [3], Beata Sznajder[1], Andy Timmins[1], Fahimeh Shahinnia[1], Zed Rengel[4], Hossein Khabaz-Saberi[4], Haydn Kuchel[2], Mamoru Okamoto[1], Peter Langridge[1]

[1]Australian Centre for Plant Functional Genomics (ACPFG), University of Adelaide, PMB1, Glen Osmond, SA 5064, Australia

[2]Australian Grain Technologies, PMB1, Glen Osmond, SA 5064, Australia

[3]School of Agriculture, Food and Wine, Waite Research Institute, University of Adelaide, PMB 1, Glen Osmond, SA 5064, Australia

[4]Soil Science and Plant Nutrition M087, School of Earth and Environment, University of Western Australia, 35 Stirling Highway, Crawley WA 6009, Australia

Present address:

Fahimeh Shahinnia

Leibniz-Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstr. 3, 06466 Gatersleben, Germany

Authors:

Ms. Saba Mahjourimajd (saba.mahjourimajd@adelaide.edu.au)

Dr. Julian Taylor (Julian.taylor@adelaide.edu.au)

Ms. Beata Sznajder (beata.sznajder@acpfg.com.au)

Dr. Andy Timmins (andy.timmins@acpfg.com.au)

Dr. Fahimeh Shahinnia (shahinnia@ipk-gatersleben.de)

Prof. Zed Rengel (zed.rengel@uwa.edu.au)

Dr. Hossein Khabaz-Saberi (Hossein.khabaz-saberi@uwa.edu.au)

Dr. Haydn Kuchel (haydn.kuchel@ausgraintech.com.au)

Dr. Mamoru Okamoto (mamoru.okamoto@acpfg.com.au)

Prof. Peter Langridge (peter.langridge@adelaide.edu.au)

Corresponding authors: Saba Mahjourimajd, e-mail: saba.mahjourimajd@adelaide.edu.au,

Peter Langridge, e-mail: peter.langridge@adelaide.edu.au

Office phone: +61 8 83137368

Fax No: +61 8 8313 7102

Abstract

Key message Selection for efficient use of soil nitrogen can be enhanced by defining the genetic control of both grain yield under variable N supply and N responsiveness.

Abstract Nitrogen (N) is a major nutrient needed to attain optimal grain yield (GY) in all environments. Nitrogen fertilisers represent a significant production cost, in terms of both the purchase price and the relevant environmental costs. Developing genotypes capable of taking up N early during development while limiting biomass production after establishment and showing high N-use efficiency (NUE) would be economically beneficial. Genetic variation in NUE has been shown previously. Here we described the genetic characterisation of NUE and identified the genetic loci underlying N response under different N fertiliser regimes in a bread wheat sub-population of doubled-haploid lines derived from a cross between two Australian genotypes (RAC875 × Kukri) bred for a similar production environment. NUE field trials were carried out at four sites in South Australia and two in Western Australia over a period of three years. There was genotype-by-environment-by-treatment interaction across the sites and also good transgressive segregation for yield under different N supply in the population. We detected some significant Quantitative Trait Loci (QTL) associated with NUE and N response at different rates of N application across the sites and years. It was also possible to identify lines showing positive N response based on the rankings of their Best Linear Unbiased Predictions (BLUPs) within a trial. Dissecting the complexity of the N effect on yield through QTL analysis represents the first stage in cloning genes underlying the loci

affecting NUE in wheat and elucidating the molecular and physiological basis of efficient use of applied N.

Keywords: wheat, selection, breeding, Nitrogen use efficiency (NUE), Best Linear Unbiased Prediction (BLUP), Quantitative trait loci (QTL)

Abbreviations

Nitrogen	N
Nitrogen use efficiency	NUE
Grain yield	GY
Responsive grain yield	RGY
Doubled haploid	DH
Quantitative trait loci	QTL
Best Linear Unbiased Prediction	BLUP
N uptake efficiency	NupE
N utilisation efficiency	NutE
Genotype	G
Single Nucleotide Polymorphism	SNP
centiMorgans	cM
Logarithm of the Odds	LOD
Composite interval mapping	CIM

Introduction

Wheat (*Triticum aestivum* L.) is the most widely grown crop globally and a major source of carbohydrates and proteins in human nutrition. Nitrogen (N) fertilisation is critical for obtaining high GY and high grain protein content. The global demand for N has been

increasing and was predicted to exceed 112 million tonnes in 2015, indicating reliance of the world food and fibre production on N input (FAO 2011). However, the increasing cost of energy is driving up the price of N fertiliser, and there are growing environmental concerns related to N pollution due to runoff and leaching. The annual consumption of N fertiliser in Australian agriculture exceeds 1 million tonnes, but varies due to climate variability and price fluctuations (ABARE 2011). Therefore, improving NUE in wheat, while maintaining high grain production, is an important target for breeders. NUE is also a high priority in low-yielding areas with a Mediterranean-type climate such as southern Australia characterised by low rainfall and high temperature during late stages in the wheat growing season.

Nitrogen use efficiency is a term with a range of meanings. Generally, NUE is calculated as the ratio of GY to N supplied and indicates how much supplied N a genotype can take up (NupE) and utilise (NutE) (Moll et al. 1982). Nitrogen use efficiency and its components; NupE and NutE, are influenced by genotypic variation, environmental factors (the interaction of climate, soil, water availability and other factors) and N management (Xu et al. 2012). Cyclic and low rainfall in many low-yielding environments may intensify the side effects of excess N and result in low NUE and GY known as haying-off (McDonald 1992). Angus and Van Herwaarden (2001) described that increased transpiration during the vegetative phase of growth (due to excessive plant vigour in response to N fertiliser) can lead to particularly inefficient water use. Increased N status can also reduce the soluble carbohydrate reserves available for re-translocation to grain after anthesis. Climate conditions, particularly rainfall amount and distribution, have an important role in N uptake and assimilation in cereals after anthesis (Ercoli et al. 2008). However, soil moisture is required both during and after vegetative growth to support N uptake.

To improve NUE, consideration needs to be given to genotypes, environmental effects, N management and the interaction of these (Raun and Johnson 1999). In order to improve wheat germplasm for NUE, plant breeders have assessed the genetic variation for NUE, genetic architecture for N traits, and G×N interaction. Previous studies revealed genetic variability for

NUE, N uptake efficiency and N utilisation efficiency in maize (Gallais and Hirel 2004) and wheat (Le Gouis 2011). It has also been important to identify genotypes showing high NUE, but also able to yield well under both high and low N supply conditions (Hirel et al. 2007). Segregating populations made from varieties differing in N response have been important to study the genetic basis of NUE and associated traits. In a multi-environment study, Cormier et al. (2013) assessed recent breeding progress on NUE in wheat and emphasised the value of improving NUE in varieties grown at low N supply to counteract the increasing cost of N fertiliser (Rothstein 2007). In addition, N management could be improved by optimising N application and synchronising crop N demand and soil N supply to lead to a significant reduction in environmental pollution and savings of money and energy.

Quantitative trait loci mapping helps provide a genetic understanding of quantitative traits and the genes controlling complex traits. Many significant QTL have been detected at high and low N in different growth conditions. For example, in wheat, An et al. (2006), Laperche et al. (2007) and Guo et al. (2012) reported significant QTL in controlled conditions, and several significant genome regions underlying NUE were detected in field trials (Quarrie et al. 2005; Fontaine et al. 2009).

Habash et al. (2007) undertook a QTL analysis for 21 traits related to growth, yield and leaf N assimilation during grain filling in hexaploid wheat using a mapping population from the cross of Chinese Spring and SQ1 (a high abscisic acid-expressing breeding line). They detected major QTL on chromosomes 2A, 4A and 6B for glutamine-synthetase (GS) activity, ear number per plant, peduncle N, grain N and GY. In a recent study by Xu et al. (2014) on mapping QTL for yield and N‐related traits in wheat, regions on chromosomes 2D, 4B, 4D, 5A (2), 6A and 7A, showed significant effects on N concentration in grain and shoots and NutE. Bordes et al. (2013) identified 54 main regions involving almost all chromosomes that influenced yield and its components, plant height, heading date and grain protein concentration. These chromosomal regions were proposed as good candidates to be used in breeding programs to improve the performance of wheat varieties at moderate N fertilisation

rates (Gupta et al. 2010) and ultimately as a resource for positional cloning of genes involved in NUE. However, the large number and variable performance of these QTL means it is unlikely breeders would actually use the information. Ideally, we require QTL that have been identified in well-adapted germplasm and show stable performance across multiple environments or known environmental responses. The present study aimed to characterise the genetic basis of N response in a population derived from a cross between two Australian genotypes bred for the same production environment. The population used for the study was derived from a cross between two highly adapted lines bred for the target environment. Therefore, QTL identified are directly relevant to breeding program. The main objectives were to study the genetic basis for variation in NUE and selection of N responsive genotypes in the low-yielding environments of southern Australia.

Materials and methods

Plant material

A split-plot design with incomplete replication was used for all experiments. Parental lines and some local genotypes were included in all NUE field trials. The mapping population consisted of 156 DH lines in the South Australia trials in 2011 and 2012, and 148 DH lines in the Western Australia trials in 2013, from a cross between RAC875 (female) and Kukri (male). The lines studied were selected from a large DH population of 324 lines to ensure that they all showed similar maturity, thereby minimising the impact of phenology (Fleury et al. 2010). The parents were both bred for the Mediterranean-type environment across southern Australia, but showed marked differences in performance under severe drought and heat stress (Bennett et al. 2012a).

Field experiments

The genotypes, grown in sub-plots, were partially replicated in the field trials at different rates of N (urea) application as the main plots (low N; no fertilisation, high N; half fertilisation and full fertilisation depending on the usual N application practice at the sites, Table 1). Soil

analyses were performed by CSBP Future Farm Analytical Laboratories (Bibra Lake, Australia, Table 1). The standard regional management practices were applied to all fields and years. GY (kg ha^{-1}) was measured for all plots at varying N applications.

SNP genotype calling

Raw intensity (.idat) files for all 322 doubled-haploid lines plus two replicates of the parents (RAC875 × Kukri) were imported into the polyploid version of GS (Wang et al. 2014) along with a custom sample-sheet and a SNP manifest file (*Wheat90k_ConsAkhunovKSU_15033654_A.bpm*). Prior to running the clustering algorithms within GS, a number of quality control checks were made. Firstly, the intensity plots for measures such as signal intensity and staining controls were manually inspected in order to ensure that the intensities fell within the normal range. Secondly, low performing samples were identified by generating scatter plots. Such samples were flagged as potential problems, but were not excluded from the cluster calling at this stage.

Cluster patterns were generated for each SNP using the semi-automated procedure described by Wang et al. (2014). At the conclusion of each step, SNPs were filtered based upon metrics such as call frequency and number of clusters. The filtered SNPs were then annotated following the published workflow. For example, at the conclusion of step 2, SNPs which exhibited a '# Clusters' metric equal to 1 were annotated as 'Monomorphic'. SNPs that did not fall within the criteria specified by the published workflow were assigned to a 'No Annotation' category. A visual examination of the cluster patterns was made and, if possible, the clusters were manually curated and the SNP annotated accordingly. From this process, there was a total of 37437 monomorphic markers, 17830 polymorphic markers and 26410 markers that exhibited multiple clusters or ambiguous cluster patterns.

Genetic linkage map

Before linkage map construction, the 63757 monomorphic markers and markers with ambiguous cluster patterns were removed and the 17830 polymorphic SNP markers across the 322 DH genotypes were diagnostically checked. Initially, three lines containing more than

20% missing values across the marker set as well as three lines, that were considered to be clones were removed. From this reduced set, 2233 markers were removed that showed significant (*p-value* < 0.05) segregation distortion patterns that deviated from the usual 1:1 allele ratio assumed for a bi-parental population. To check the quality of the remaining SNP marker set, an initial linkage map was constructed using the MSTmap algorithm (Wu et al. 2008) integrated into the linkage map construction functions of the R/ASMap package (Taylor and Butler 2014) available in the R Statistical Computing Environment (R Development Core Team 2014). From this initial map the genotypes were checked across the complete genome and a total of 82 lines were removed that exhibited excessive recombination counts.

The complete set of 17830 polymorphic SNP markers for the 234 lines was then integrated with the 226 matching genotypes of the single sequence repeat (SSR) and DArTs markers from the RAC875 × Kukri genetic linkage map described in Bennet et al (2012a). Prior to integration, markers in the SSR-DArTs linkage map containing more than 20% missing values were removed. The integrated SSR-DArTs-SNP marker set contained a total of 18333 markers across 226 genotypes, Marker segregation distortion was checked again and 2340 markers were removed. With the remaining 15993 markers an initial map was constructed using the MSTmap linkage map construction functions of R/ASMap. A further eight lines were removed due to excessive recombination counts, and the map was re-constructed a final time. Linkage groups with less than ten markers were deemed to be unlinked and omitted from further construction. Linkage group assignment and orientation was determined through a comparison of the remaining 408 SSR-DArT markers in the newly constructed linkage map with the SSR-DArT linkage map of Bennet et al. (2012a) as well as a comparison of SNP markers to the 90K SNP array based wheat consensus map. After this process, one linkage group remained unassigned, while two pairs of linkage groups and one set of three linkage groups were merged. The final integrated SSR-DArTs-SNP linkage map consisted of 218 individuals with 15911 markers assigned to 26 linkage groups. After

removing co-located markers this was reduced to 1333 unique loci with a total map length of 2864.3 cM and average interval distance of 2.18 cM (minimum = 0.1 cM and maximum = 48.1 cM).

Statistical analysis

Linear mixed model analysis

Analysis of GY was conducted using a multi-treatment-environment trial (MTET) linear mixed model that appropriately captured genetic and non-genetic sources of variation present across the multiple treatments and environments (Smith et al. 2001; Smith et al. 2005). For each treatment by environment the fixed component of the MTET model contained a factor that consisted of one level for the complete set of DH lines and a level for each of the parents and controls. The inclusion of this term ensured that the parents and controls remained fixed in the analysis and did not contribute to the genetic variation of the DH lines in any treatment by environment combination. In addition, for each treatment by environment combination the fixed component also contained phenology genes *ppdB1* and *ppdD1* as numerical covariates (Bonneau et al. 2012) as well as modelled linear trends possibly existing across the row and ranges of the environment. Extraneous non-genetic sources of design variation, such as blocks or bays, were captured using independent random effects. For each of the environment specific residuals a separable AR1 × AR1 (AR1 = autoregressive process of order 1) process was used to adequately account for spatial correlation of GY measurements induced by the rectangular layout of the experiment.

An important component of the MTET model was the inclusion of a random effects term to model the variance-covariance structure for the genotype by treatment by environment (GTE) interaction. This structure consisted of a genetic variance of the DH lines for each treatment within an environment as well as covariances or correlations that reflect the genetic relationship of the DH lines between varying levels of N within and between environments. Due to the large number of treatment by environment combinations, this genetic random

effects term was parsimoniously approximated by a Factor Analytic model (Smith et al. 2001; Smith et al. 2005).

After fitting the MTET model, the GY BLUPs for the DH genotypes were extracted for all levels of the N treatment within each of the environments. For any two levels of N in an environment the responsiveness GY (RGY) BLUPs for the DH genotypes were determined by extracting the residuals from the random regression of the GY BLUPs for the DH genotypes at the high level of the N treatment on the GY BLUPs for the DH genotypes at the low level of the treatment. The random regression line therefore represents the average performance of a DH genotype for the two N levels. Positive residuals from this regression indicate a genotype responded well on average to the high application of N and conversely a genotype with negative residuals indicated a poor responsiveness on average. Each two treatment combinations can then be viewed as having a GY BLUP that is equivalent to the DH genotype BLUPs for the lower level of the N treatment and RGY BLUP that is equivalent to the genetic response of the DH genotypes to the application of the higher level of the N treatment given the lower level of N.

For each N treatment by environment combination broad sense heritabilities were calculated using the formula derived in Cullis et al. (2006). All statistical modelling was conducted using the flexible linear mixed modelling package ASReml-R (Butler et al. 2009) available in the R statistical computing environment (R Development Core Team 2014).

QTL mapping

Using the 1333 unique loci of the integrated SSR-DArTs-SNP linkage map, QTL analyses were conducted on the GY BLUPs of the DH genotypes for each treatment by environment combination as well as the RGY BLUPs derived from each two level N treatment combination within each environment. The QTL analyses used the CIM approach implemented in WinQTLCart-version 2.5 (Model 6 standard analysis) (Wang et al. 2007). LOD value thresholds were determined with 1000 fold permutations (Churchill and Doerge 1994) and a family wise error rate $P = 0.05$. This corresponded to a minimum LOD score of

2.9. Trait abbreviations and QTL designations follow the nomenclature suggested in the wheat catalogue of gene symbols (McIntosh et al. 2003) with 'asw' signifying 'Australian Spring Wheat'. Significant QTL were summarised with their position on a linkage group and LOD score as well as their contribution to the genetic variance.

Results

156 DH lines at South Australia, and 148 DH lines at the Western Australia, of RAC875 and Kukri was studied to identify significant genetic factors underlying NUE based on GY. Average GY ranged from 1,805 kg ha^{-1} at YAN 11 to 3,065 kg ha^{-1} at ED 13 (Table 1). Under low N compared to high N conditions, yield was reduced by 15% in PIN 12 and 25% in LAM 12 in the population. Parental lines showed different trends for yield performance at different N fertilisation across sites (Fig. 1). For instance, at PIN 12, the parents were significantly different at both high and low rates of N, but at other sites the difference was not significant. In addition, at YAN 11, the parents showed no response to increasing N. The variation for GY among the DH lines exceeded the parental lines (Table 2 and Fig. 2) demonstrating significant transgressive segregation in the population. In the initial stages of fitting the GY MTET linear mixed model it was discovered there was no significant genetic variation of the DH genotypes for the 18 kg ha^{-1} of N at LAM 12. For this reason it was excluded from further linear mixed model analysis. The final MTET model incorporated an FA model of order 4 for the GTE interaction spanning all N treatment levels across the sites in South Australia and Western Australia. The estimated genetic correlation matrix was extracted from the model and presented in Table 3. The table indicates there is moderate to strong genetic correlation between varying levels of N within and between South Australian trial sites. Similarly, there are also strong genetic correlations between the two levels of N within and between the Western Australian sites. Table 3 also indicates that the varying levels of N at the South Australian sites have weak or negligible genetic correlation with the two levels of N at the Western Australian sites. Broad sense heritability for yield in PIN 11 was highest (0.90) at 75

kg ha^{-1} N fertiliser, while it was very low at LAM 12 in all N treatments (Supplementary Table 1).

The GY BLUPs for the DH genotypes were then extracted from the final MTET model and RGY BLUPS for the DH genotypes were calculated for all two N treatment combinations within each environment. For example, in PIN 12, GY BLUPs of the DH genotypes for N0, N75 and N150 kg ha^{-1} were extracted from the model and used to form RGY BLUPs for the DH genotypes denoted N75-N0, N150-N0 and N150-N75, where, for example, N150-N0 represents the response of the DH genotypes to the application of 150 kg ha^{-1} of N given the BLUPs for the DH genotypes at 0 kg N ha^{-1}. Fig. 3 presents a two dimensional scatter plot of the GY BLUPs for the DH genotypes against their RGY BLUPs for all available two N treatment combinations within an environment. To aid interpretation, each panel was divided into four sub-areas or quadrants. For example, the upper right quadrant (Q1) indicates DH lines that show above average GY and response to the application of N whereas those in the lower left hand quadrant (Q3) indicate below average GY and N response.

To assess the individual genetic performance of DH lines across environments and two level N treatment combinations, a two-dimensional ranking scheme was developed using the GY and RGY BLUPs. Preceding this development, to ensure an equal weighting, each of pair of GY and RGY BLUPs were independently standardised. Additionally, due to their moderate to strong genetic correlation, only the South Australian trials were used in this assessment. To aid in the description of the ranking scheme, Fig. 4 shows the top five ranked varieties in the upper half of the figure as well as the bottom five varieties in the lower half. The length of each line and the proximity of the line to the optimal 45 degree angle provides a measure of the characteristics of the DH genotype for each two level N treatment combination in an environment. Across all two level N treatment combinations and environments, a DH genotype is then ranked by summing the angle differences to the optimal 45 degree line and dividing by the mean of the line lengths. Using this ranking scheme, DH_R214 was the best performing line and showed above average GY and N response in 9 of the 10 two level N

treatment combinations across environments, while DH_R241 was the poorest performing line with below average GY and N response in 9 of the 10 two level N treatment combinations across environments.

QTL associated with GY

In total, 29 significant QTL for GY were identified, including 17 GY-QTL on chromosomes 1A, 1B, 2A, 3D-2, 4A, 4B, 4D, 5A, 6A, 7A-1, 7B and 7D and 12 QTL for grain yield response (GY at high N – GY at low N) across all treatments and environments (Supplementary Fig. 1). The highest LOD was 15.4 on chromosome 2A with the largest additive effect detected for GY at PIN 11, and the highest proportion of genotypic variation explained (27 %), (Table 4). The only QTL that was specific for GY at low N, *QYLD.asw-7B*, explained only 5% of the genotypic variance. The allele from Kukri, within the interval *CAP12_c1816_325 − Kukri_c109962_396* on 7B, was responsible for an improvement in GY. There was also one QTL on 6A under high N application carrying the positive allele from RAC875 for increased GY detected only at PIN 11. The rest of QTL were detected at both high and low N trials, with the contributions coming from of both parents, showing more QTL at high N.

Among the 17 GY-QTL, there were nine site-specific QTL that accounted for a relatively high proportion of the genetic variation (Table 4). These included three QTL on 4A, 4D and 7D recorded at both low and high N in YAN 11, ED 13 and PIN 12, and one on 6A at high N, and another one on 7B at low N. However, both Kukri (2A, 4A, 4B, 4D, 5A, 7B and 7D) and RAC875 (1A, 6A and 7A-1), along with a shared locus on 1B and two loci (one from each parent) on 3D-2 contributed to improving GY. The most significant QTL was on 2A and was recorded at four sites and all levels of N application. No QTL were detected on 2B and 2D where the *Ppd-B1* and *Ppd-D1* loci are located, confirming that the data had been adequately adjusted for these maturity effects.

QTL for N response

The responsiveness of DH lines to N application was assessed by comparing yields at different levels of N application to generate and score for responsive GY, RGY (Table 5). For the response to the rate of N fertilisation, 12 QTL were detected, with the predominant proportion of desirable alleles coming from the Kukri parent. These QTL were on chromosomes 1A, 1B, 2A, 2B, 3B, 3D-2 (two loci), 5A, 6A, 6B, 7A-2 and 7B with a LOD range of 3 to 11.8. All sites revealed loci that showed a differential response to the rate of N application. Nine RGY-QTL, were classified as adaptive QTL since they were detected at only one site. These were located on chromosomes 1A, 1B, 2B, 3B, 3D-2 (two loci), 5A, 6B and 7A-2. *QRGY.asw-2A* explained the highest proportion of variance (R^2 = 20 %) and was stable across three sites. Further, two putative QTL on 3D-2, delineated by markers *cfd0064 − Excalibur_c3510_1888* and *RAC875_rep_c79167_809 − CAP12_c1384_314,* were associated with RGY at South Australia and Western Australia.

Several RGY regions were also detected in the GY mapping study. These included the regions on 1A, 1B, 2A, 3D-2, 5A, 6A and 7B. Although the same regions were detected, they were not necessarily detected from the same trials; for example, the 1A RGY locus appeared in the PIN 12 trial and the same region was detected in the other sites for GY data. Similarly, the 3D-2, 6A and 7B RGY loci were detected in different trials for GY data (Tables 4 and 5). Two QTL were detected for GY and RGY on 3D-2, but only the locus at 18.7 cM was common. The 2A, 6A and 7B RGY loci appear to show contribution of both parents depending on the trial, but this may actually reflect two separate but closely linked loci given that the QTL peaks were slightly shifted. The RGY regions (2B, 3B, 6B and 7A-2) were not detected in the GY analysis and are therefore assumed to have no major effect on yield per se.

Discussion

In this study GY under different rates of N application was measured across multiple sites giving a total of 16 N×E treatments (four sites at three N rates in South Australia in 2011-2012 and two sites and two N treatments in Western Australia in 2013). The study used a

population developed from two lines that had been bred for the same production environment, but with different genetic backgrounds. This means that many key albeit well known adaptive traits had already been optimised in the parents (such as plant height and maturity).

An important aspect of this study was the focus on field performance in the low-yielding, Mediterranean-type environments found in southern Australia. In these environments strong vegetative growth, in response to abundant N early in the growing season, can negatively impact yield due to increased water loss late in the season during flowering and grain filling (Mahjourimajd et al. 2015). Well-adapted plants are expected to be efficient in N uptake during vegetative growth, maintain optimal vegetative biomass and only mobilise N late in development. This contrasts to previous studies that have been conducted in relatively high yielding environments where large early biomass is associated with increased GY (Reynolds et al. 2012).

Heritability and genetic variability tended to be lowest at the low N treatment, consistent with previous studies (Brancourt-Hulmel et al. 2005; Laperche et al. 2006; Cormier et al. 2013). The QTL on chromosomes 1A, 1B, 2A, 3D-2 and 7A-1 for GY and 2A for RGY were detected at three locations at least and represent the most stable QTL from this study (Tables 4 and 5). These genomic regions are the best candidates for more extensive NUE studies and for positional cloning of the gene(s) underlying the QTL. The remaining QTL were only detected at one or two sites. These site-specific or unstable QTL reflect regions associated with adaptation to specific environmental conditions rather than the level of applied N alone (Loudet et al. 2003). Overall maturity effects were effectively managed in these experiments. However, the regions controlling maturity are presented in Supplementary Tables 2 and 3.

The magnitude and direction of allelic effects across QTL showed that both parents could contribute to increased yield. This observation also helps explain the strong transgressive segregation seen across the population. Although both parents contributed desirable alleles, Kukri alleles predominated. The QTL on chromosomes 4A, 4B, 4D, 7A-1 and 7D were associated only with GY and were essentially independent of the N response. Conversely, the

RGY QTL on chromosomes 2B, 3B, 6B and 7A-2 led to increased yield, making these the ideal targets for enhancing NUE in improvement programs.

Some QTL detected in this study do require more detailed analysis. For example, the region close to marker *RAC875_rep_c104986_200 − RAC875_c11899_366* on 1A showed a major effect on yield under low and high rates of N application at three sites, PIN 11, ED 13 and YAN 11, and is adjacent to a RGY-QTL identified at PIN 12. It seems probable that these QTL are the same, and we are in reality seeing an effect of the QTL in three separate trials. However, this needs to be tested. Importantly, these QTL regions would appear to represent a region where both N response and GY are controlled and where significant genetic gain for NUE could be achieved. In a study with the same population, Bennett et al. (2012b) identified QTL for GY on chromosomes 1A, 1B, 2A, 2B, 2D, 4D, 6D and 7A. Among these QTL, regions on 1A, 1B, 2A, 4D and 7A-1 were detected in research presented here for the GY-QTL and also on 2B for RGY. The genomic regions controlling N response were detected on all homoeologous chromosome groups, but the A and B genomes predominated. This observation is consistent with the results demonstrated by Bogard et al. (2011).

Many QTL for NUE and related traits have been described in wheat (An et al. 2006; Habash et al. 2007; Laperche et al. 2007; Fontaine et al. 2009). Bogard et al. (2011) detected QTL on 2D, 3B, 5A, 6B, 7A, 7B, 7D in wheat grown at various N fertilisation rates. They also identified that several NUE regions co-located with QTL for grain protein content on chromosomes 2D, 3B, 5A, 7D. Similarly, Bordes et al. (2013) showed large variability in response of grain yield to N fertilisation and detected major QTL using different measures of NUE such as the difference between yield under high N versus low N (HN–LN), the ratio of yield under high N relative to low N (LN/HN) and the joint regression. They demonstrated significant regions for both GY and RGY on 1D, 2D, 3B and 5B and also for GY on 3D and for RGY on 5D. Xu et al. (2014) detected major QTL on 2D, 4B, 6A and 7A for yield components under different N supplement regimes. In their research, NUE was studied by

assessing response of GY-related traits to N fertilisation. Among these, there are some overlapped regions with the identified loci in this study.

Several of the QTL presented here are co-located with other known QTL. For example, *QYLD.asw-1B* was detected for GY at the three sites in South Australia and West Australia. This QTL was close to the region identified for a GY-QTL by Quarrie et al. (2005). Guo et al. (2012) also reported chromosome 1B to be associated with both N uptake and utilisation in wheat.

Different growth conditions in South Australia and Western Australia are likely to have caused some of the instability detected in the present QTL study. However, regions on 1A, 1B and 3D-2 for GY and also on 3D-2 for RGY were detected at both South Australia and Western Australia.

In addition to the identification of QTL associated with GY and RGY, the present study allowed the classification of individual lines in the population considering their genetic yield and responsiveness to N fertilisation (Figs 3 and 4). The most valuable lines for breeding are those that consistently showed both a high yield and a strong response to N. In contrast to most previous studies on NUE in wheat, the parents used to develop the populations are well-adapted and commercially relevant. Consequently, their progeny are directly relevant to breeders. The consistent high-yielding/high N response lines identified in this study have now been provided to breeding programs for further development.

From a research perspective the lines that showed a consistent low N response and low yield are also of interest. These lines can be compared with the high yielding/high N response lines in biochemical and physiological studies to determine the basis for the difference in performance and help improve screening and evaluation methods.

Conclusion

Significant genetic variation for GY was documented at varying rates of N application. The number of QTL detected at each trial was variable, but some loci were seen across multiple trials. These loci would offer greatest benefit to breeders in selecting for improved NUE.

In addition to identifying key regions associated with NUE that could be used to track and move the desirable alleles into breeding programs, this study has identified good target regions for a more detailed molecular analysis and ultimately cloning of the genes underlying the N response. The analysis allowed us to separate the relationship between yield and N supply, and also to differentiate N responsiveness of individual lines. Some QTL detected were common to both GY and RGY and will be good targets for more detailed physiological analysis. Lines that show a strong response to the rate of applied N are not necessarily high yielding. However, we identified lines that were both high yielding and highly N-responsive in multiple trials. These lines represent particularly attractive material for further crossing and selection given that both parents are well-adapted lines.

Acknowledgements

This research was funded through grants from the Australian Research Council, the Grains Research and Development Corporation, the Government of South Australia and the University of Adelaide.

Reference

ABARE (2011) Australian Bureau of Agricultural and Resource Economics and Sciences. http://www.agriculture.gov.au/abares.

An D, Su J, Liu Q, Zhu Y, Tong Y, Li J, Jing R, Li B, Li Z (2006) Mapping QTLs for nitrogen uptake in relation to the early growth of wheat (*Triticum aestivum* L.). Plant Soil 284:73-84

Angus J, Van Herwaarden A (2001) Increasing water use and water use efficiency in dryland wheat. Agron J 93:290-298

Bennett D, Izanloo A, Edwards J, Kuchel H, Chalmers K, Tester M, Reynolds M, Schnurbusch T, Langridge P (2012a) Identification of novel quantitative trait loci for days to ear emergence and flag leaf glaucousness in a bread wheat (*Triticum aestivum* L.) population adapted to southern Australian conditions. Theor Appl Genet 124:697-711

Bennett D, Izanloo A, Reynolds M, Kuchel H, Langridge P, Schnurbusch T (2012b) Genetic dissection of grain yield and physical grain quality in bread wheat (*Triticum aestivum* L.) under water-limited environments. Theor Appl Genet 125:255-271

Bogard M, Jourdan M, Allard V, Martre P, Perretant MR, Ravel C, Heumez E, Orford S, Snape J, Griffiths S (2011) Anthesis date mainly explained correlations between post-anthesis leaf senescence, grain yield,

and grain protein concentration in a winter wheat population segregating for flowering time QTLs. J Exp Bot 62:3621-3636
Bonneau J, Taylor J, Parent B, Reynolds M, Feuillet C, Langridge P, Mather D (2012) Mult-environment analysis and fine mapping of a yield related QTL on chromosome 3B of wheat. Theoretical and Applied Genetics, Accepted:747-761
Bordes J, Ravel C, Jaubertie J, Duperrier B, Gardet O, Heumez E, Pissavy A, Charmet G, Le Gouis J, Balfourier F (2013) Genomic regions associated with the nitrogen limitation response revealed in a global wheat core collection. Theor Appl Genet 126:805-822
Brancourt-Hulmel M, Heumez E, Pluchard P, Beghin D, Depatureaux C, Giraud A, Le Gouis J (2005) Indirect versus direct selection of winter wheat for low-input or high-input levels. Crop Sci 45:1427-1431
Butler D, Cullis B, Gilmour A, Gogel B (2009) ASReml-R reference manual release, 3 edn. Queensland Department of Primary Industries. Technical report,
Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138:963-971
Cormier F, Faure S, Dubreuil P, Heumez E, Beauchêne K, Lafarge S, Praud S, Le Gouis J (2013) A multi-environmental study of recent breeding progress on nitrogen use efficiency in wheat (*Triticum aestivum* L.). Theor Appl Genet 126:3035-3048
Cullis BR, Smith AB, Coombes NE (2006) On the design of early generation variety trials with correlated data. J Agric Biol Environ Stat 11:381-393
Ercoli L, Lulli L, Mariotti M, Masoni A, Arduini I (2008) Post-anthesis dry matter and nitrogen dynamics in durum wheat as affected by nitrogen supply and soil water availability. Eur J Agron 28:138-147
FAO (2011) Current world fertilizer trends and outlook to 2015. ftp://ftp.fao.org/ag/agp/docs/cwfto15.pdf.
Fleury D, Jefferies S, Kuchel H, Langridge P (2010) Genetic and genomic tools to improve drought tolerance in wheat. J Exp Bot 61:3211-3222
Fontaine J-X, Ravel C, Pageau K, Heumez E, Dubois F, Hirel B, Le Gouis J (2009) A quantitative genetic study for elucidating the contribution of glutamine synthetase, glutamate dehydrogenase and other nitrogen-related physiological traits to the agronomic performance of common wheat. Theor Appl Genet 119:645-662
Gallais A, Hirel B (2004) An approach to the genetics of nitrogen use efficiency in maize. J Exp Bot 55:295-306
Guo Y, Kong F-m, Xu Y-f, Zhao Y, Liang X, Wang Y-y, An D-g, Li S-s (2012) QTL mapping for seedling traits in wheat grown under varying concentrations of N, P and K nutrients. Theor Appl Genet 124:851-865
Gupta P, Kumar J, Mir R, Kumar A (2010) Marker-Assisted Selection as a Component of Conventional Plant Breeding. Plant Breed Rev 33:145-217
Habash DZ, Bernard S, Schondelmaier J, Weyen J, Quarrie SA (2007) The genetics of nitrogen use in hexaploid wheat: N utilisation, development and yield. Theor Appl Genet 114:403-419
Hirel B, Le Gouis J, Ney B, Gallais A (2007) The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. J Exp Bot 58:2369-2387
Laperche A, Brancourt-Hulmel M, Heumez E, Gardet O, Gouis JL (2006) Estimation of genetic parameters of a DH wheat population grown at different N stress levels characterized by probe genotypes. Theor Appl Genet 112:797-807
Laperche A, Brancourt-Hulmel M, Heumez E, Gardet O, Hanocq E, Devienne-Barret F, Le Gouis J (2007) Using genotype× nitrogen interaction variables to evaluate the QTL involved in wheat tolerance to nitrogen constraints. Theor Appl Genet 115:399-415
Le Gouis J (2011) Genetic Improvement of Nutrient Use Efficiency in Wheat" in Molecular basis of nutrient use efficiency incrops First edn. John Wiley, Oxford
Loudet O, Chaillou S, Merigout P, Talbotec J, Daniel-Vedele F (2003) Quantitative trait loci analysis of nitrogen use efficiency in Arabidopsis. Plant Physiol 131:345-358
McDonald G (1992) Effects of nitrogenous fertilizer on the growth, grain yield and grain protein concentration of wheat. Aust J Agric Res 43:949-967
McIntosh R, Yamazaki Y, Devos K, Dubcovsky J, Rogers W, Appels R Catalogue of gene symbols for wheat. In: 10th International Wheat Genetics Symposium. Paestum (Italie), 2003.
Moll RH, Kamprath EJ, Jackson WA (1982) Analysis and interpretation of factors which contribute to efficiency of nitrogen-utilization. Agron J 74:562-564
Quarrie S, Steed A, Calestani C, Semikhodskii A, Lebreton C, Chinoy C, Steele N, Pljevljakusić D, Waterman E, Weyen J (2005) A high-density genetic map of hexaploid wheat (*Triticum aestivum* L.) from the cross Chinese Spring× SQ1 and its use to compare QTLs for grain yield across a range of environments. Theor Appl Genet 110:865-880
R Development Core Team (2014) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, ISBN: 3-900051-07-0.
Raun WR, Johnson GV (1999) Improving nitrogen use efficiency for cereal production. Agron J 91:357-363
Reynolds M, Foulkes J, Furbank R, Griffiths S, King J, Murchie E, Parry M, Slafer G (2012) Achieving yield gains in wheat. Plant Cell Environ 35:1799-1823

Rothstein SJ (2007) Returning to our roots: making plant biology research relevant to future challenges in agriculture. Plant Cell 19:2695-2699
Smith A, Cullis B, Thompson R (2001) Analyzing variety by environment data using multiplicative mixed models and adjustments for spatial field trend. Biometrics 57:1138-1147
Smith A, Cullis BR, Thompson R (2005) The analysis of crop cultivar breeding and evaluation trials: an overview of current mixed model approaches. J Agric Sci 143:449-462
Taylor J, Butler D (2014) ASMap: Linkage map construction using the MSTMap algorithm. R package version 0.3-2. http://CRAN.R-project.org/package=ASMap.
Wang S, Basten C, Zeng Z (2007) Windows QTL cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC
Wang S, Wong D, Forrest K, Allen A, Chao S, Huang BE, Maccaferri M, Salvi S, Milner SG, Cattivelli L (2014) Characterization of polyploid wheat genomic diversity using a high-density 90 000 single nucleotide polymorphism array. Plant Biotechnol J 12:787-796
Wu Y, Bhat PR, Close TJ, Lonardi S (2008) Efficient and accurate construction of genetic linkage maps from the minimum spanning tree of a graph. PLoS genetics 4:e1000212
Xu G, Fan X, Miller AJ (2012) Plant nitrogen assimilation and use efficiency. Annu Rev Plant Biol 63:153-182
Xu Y, Wang R, Tong Y, Zhao H, Xie Q, Liu D, Zhang A, Li B, Xu H, An D (2014) Mapping QTLs for yield and nitrogen-related traits in wheat: influence of nitrogen and phosphorus fertilization on QTL expression. Theor Appl Genet 127:59-72

Table 1 The location, climatic and basic soil characteristics, growing conditions and average grain yield (GY, kg ha^{-1}) of the South Australia (SA) and Western Australia (WA) in 2011-2013

Site	Year	Abbreviation	Lat[a] (°S)	Lon[b] (°E)	Elv[c] (m)	Total rain[d] (mm)	Hot day[e] (d)	Soil texture[f]	pH level ($CaCl_2$)	pH level (H_2O)	NH_4^+ nitrogen (mg kg^{-1})	NO_3^- nitrogen (mg kg^{-1})	N fertiliser rates (kg ha^{-1})	Average grain yield (kg ha^{-1})
PINERY-SA	2011	PIN 11	34.2	138.6	260	165	16	Clay	7.6	8.2	3	36	0− 75− 150	2236
YANCO-SA	2011	YAN 11	34.6	146.4	164	221	22	n.a.	n.a.	n.a.	n.a.	n.a.	0− 75− 150	1805
LAMEROO-SA	2012	LAM 12	35.3	140.5	99	144	15	Loamy	8.2	9	2	8	18− 52− 87	2007
PINERY-SA	2012	PIN 12	34.2	138.6	260	185	23	Clay	7.7	8.5	3	54	0− 75− 150	2112
ESPERANCE DOWN-WA	2013	ED 13	33.6	121.8	158	293	8	Loamy-sand	5.7	6.3	3	25	0− 60	3065
WONGAN HILLS- WA	2013	WH 13	30.8	116.7	305	163	26	Loamy-sand	6.5	6.9	4	22	0− 35	2559

n.a. data not available

[a] Latitude (Lat °S)
[b] Longitude (Lon °E)
[c] Elevation above sea level (Elv, m)
[d] Total rainfall during growth season
[e] Number of growth season hot days with temperature higher than 30 °C
[f] Soil characteristics at top 10 cm depth of soil before fertilisation

Table 2 Phenotypic performance of RAC875 × Kukri population for grain yield across Australian sites

Site and year	Parents		Doubled haploid population		
	RAC875	Kukri	Mean	Max	Min
PIN 11	2468	2370	2229	3279	593
YAN 11	1768	1893	1802	2651	795
LAM 12	1946	2200	2001	3724	739
PIN 12	2345	2012	2108	3253	871
ED 13	3151	3141	3003	4477	1472
WH 13	2683	2486	2475	3538	1316

Maximum and minimum values for the population were calculated across all nitrogen fertilisation rates

Table 3 Genetic correlation coefficients within sites for grain yield for all genotypes studied, parental lines and doubled haploid lines in nitrogen use efficiency field trials in South Australia

Site and year	Nitrogen fertilisation (kg ha^{-1})	PIN 11 0	PIN 11 75	PIN 11 150	YAN 11 0	YAN 11 75	YAN 11 150	LAM 12 52	LAM 12 87	PIN 12 0	PIN 12 75	PIN 12 150	ED 13 0	ED 13 60	WH 13 0
PIN 11	75	0.95													
PIN 11	150	0.91	0.94												
YAN 11	0	0.64	0.70	0.69											
YAN 11	75	0.68	0.73	0.73	0.99										
YAN 11	150	0.52	0.58	0.57	0.98	0.95									
LAM 12	52	0.43	0.47	0.45	0.57	0.55	0.56								
LAM 12	87	0.18	0.20	0.17	0.24	0.21	0.27	0.19							
PIN 12	0	0.39	0.36	0.35	0.42	0.39	0.57	0.27	0.22						
PIN 12	75	0.34	0.30	0.31	0.40	0.40	0.60	0.21	0.13	0.86					
PIN 12	150	0.65	0.62	0.62	0.46	0.41	0.53	0.32	0.15	0.88	0.85				
ED 13	0	0.14	0.13	0.06	-0.13	-0.20	-0.08	0.07	0.24	0.35	0.13	0.10			
ED 13	60	0.06	0.06	-0.02	-0.18	-0.26	-0.12	0.05	0.25	0.33	0.09	0.03	0.82		
WH 13	0	0.10	0.08	0.01	-0.33	-0.38	-0.30	-0.04	0.18	0.34	0.13	0.10	0.80	0.84	
WH 13	35	0.06	0.02	-0.04	-0.41	-0.45	-0.38	-0.09	0.14	0.36	0.16	0.12	0.79	0.82	0.90

Table 4 Genome regions underlying the single effect of nitrogen (N) on grain yield-BLUPs, adjoining markers (closest in bold), peak position (cM), logarithm of odd (LOD), R^2 (as %) and additive allele in trials at various Australian sites

Chr.	QTL	N effect	Site and year	Adjoining markers	Position (cM)	LOD	R^2 (%)	Allele effect
1A	1	N52	LAM 12	***Excalibur_c44711_453*** − *Excalibur_c11941_675*	17.1	2.9	6	0.02
	2	N150	PIN 11	***RAC875_rep_c104986_200*** − *RAC875_c11899_366*	43.7	5.8	9	0.1
		N0	PIN 11	*RAC875_rep_c104986_200* − ***RAC875_c11899_366***	45.7	6.4	10	0.07
		N75	PIN 11	*RAC875_rep_c104986_200* − ***RAC875_c11899_366***	45.7	6.7	10	0.12
		N0	YAN 11	*RAC875_rep_c104986_200* − ***RAC875_c11899_366***	45.7	3.8	6	0.06
		N75	YAN 11	*RAC875_rep_c104986_200* − ***RAC875_c11899_366***	45.7	4.4	7	0.05
		N0	ED 13	***RAC875_c11899_366*** − *wsnp_Ra_c20126_29372577*	46.7	3.9	8	0.05
		N60	ED 13	***RAC875_c11899_366*** − *wsnp_Ra_c20126_29372577*	46.7	3.5	7	0.07
		N35	WH 13	***RFL_Contig3715_263*** − *gwm0357*	48.9	3.7	8	0.06
1B	3	N75	YAN 11	*wsnp_Ex_rep_c66980_65419811* − ***Kukri_c1529_462***	104.1	3.3	5	0.04
		N0	ED 13	***barc0207*** − *wsnp_Ex_c23992_33235984*	116.8	3.9	8	-0.05
		N60	ED 13	***barc0207*** − *wsnp_Ex_c23992_33235984*	116.8	3.6	7	-0.07
	4	N0	WH 13	*wsnp_Ex_rep_c66255_64400455* − ***barc0256***	137	3.6	8	-0.06
		N35	WH 13	*wsnp_Ex_rep_c66255_64400455* − ***barc0256***	137	3.9	9	-0.06
2A	5	N0	PIN 11	*BS00011893_51* − ***Kukri_c46040_620***	26.7	14.4	25	-0.11
		N75	PIN 11	*BS00011893_51* − ***Kukri_c46040_620***	26.7	15.4	27	-0.2
		N150	PIN 11	*BS00011893_51* − ***Kukri_c46040_620***	26.7	16	28	-0.18
		N0	YAN 11	*BS00011893_51* − ***Kukri_c46040_620***	26.7	9.8	18	-0.1
		N75	YAN 11	*BS00011893_51* − ***Kukri_c46040_620***	26.7	11.2	19	-0.08
		N150	YAN 11	*BS00011893_51* − ***Kukri_c46040_620***	26.7	7.7	15	-0.07
		N52	LAM 12	*BS00011893_51* − ***Kukri_c46040_620***	26.7	5.9	12	-0.04
	6	N150	PIN 12	*D_GB5Y7FA02HSMR1_278* − ***BobWhite_rep_c64012_389***	40.8	6.5	14	-0.04
3D2	7	N0	PIN 11	*cfd0064* − ***Excalibur_c3510_1888***	18.7	5.4	8	-0.06
		N75	PIN 11	*cfd0064* − ***Excalibur_c3510_1888***	18.7	6.9	10	-0.12

Chr.	QTL	N effect	Site and year	Adjoining markers	Position (cM)	LOD	R^2 (%)	Allele effect
3D2		N150	PIN 11	*cfd0064* − ***Excalibur_c3510_1888***	18.7	6.7	10	-0.11
		N75	YAN 11	*cfd0064* − ***Excalibur_c3510_1888***	18.7	5.6	9	-0.06
	8	N60	ED 13	***RAC875_c35801_905*** − *wsnp_Ex_rep_c101732_87042471*	25.9	4.9	10	0.08
4A	9	N0	YAN 11	*Excalibur_c11047_1145* − ***BS00064523_51***	145.4	3.4	6	-0.06
		N75	YAN 11	*Excalibur_c11047_1145* − ***BS00064523_51***	145.4	4.5	7	-0.05
		N150	YAN 11	*Excalibur_c11047_1145* − ***BS00064523_51***	145.4	3.7	7	-0.05
4B	10	N60	ED 13	*BS00004727_51* − ***RFL_Contig5846_1610***	79.4	4	8	-0.07
		N0	WH 13	*BS00004727_51* − ***RFL_Contig5846_1610***	79.4	4.9	11	-0.07
		N35	WH 13	*BS00068539_51* − ***BobWhite_c4818_173***	83.1	3.7	8	-0.06
4D	11	N0	ED 13	*wsnp_Ex_rep_c107564_91144523* − ***wsnp_Ku_rep_c109720_94223856***	1.8	4.4	9	-0.05
		N60	ED 13	*wsnp_Ex_rep_c79748_75305162* − ***wsnp_BF473052D_Ta_2_1***	3.3	4.1	9	-0.07
5A	12	N150	YAN 11	***BS00022867_51*** − *BS00081951_51*	177.8	3.3	5	-0.04
		N0	PIN 12	***BS00022867_51*** − *BS00081951_51*	177.8	4.5	9	-0.03
		N75	PIN 12	***BS00022867_51*** − *BS00081951_51*	177.8	3.8	8	-0.04
6A	13	N75	PIN 11	*wsnp_Ex_c2389_4479352* − ***barc0353b***	69.5	4	6	0.09
		N150	PIN 11	*wsnp_Ex_c2389_4479352* − ***barc0353b***	67.5	4.5	7	0.09
7A1	14	N75	PIN 12	*wPt.8399* − ***Excalibur_c12996_775***	82.7	4.3	9	0.04
		N0	PIN 12	***BobWhite_rep_c49790_351*** − *BobWhite_c16317_641*	85.1	4.5	9	0.03
	15	N0	YAN 11	*Excalibur_c49272_174* − ***wPt.5558***	114.4	6.1	11	0.08
		N75	YAN 11	*Excalibur_c49272_174* − ***wPt.5558***	114.4	4.7	7	0.05
		N150	YAN 11	*Excalibur_c49272_174* − ***wPt.5558***	114.4	6.2	11	0.06
		N52	LAM 12	*wPt.5558* − ***Ra_c114158_328***	116.4	4.2	9	0.03
7B	16	N0	PIN 11	*CAP12_c1816_325* − ***Kukri_c109962_396***	13.4	3.4	5	-0.05
7D	17	N0	PIN 12	*BobWhite_rep_c57051_479* − ***Ku_c884_1017***	76.6	4.3	9	-0.03
		N75	PIN 12	*BobWhite_rep_c57051_479* − ***Ku_c884_1017***	76.6	3.5	7	-0.04
		N150	PIN 12	***Kukri_c100613_331*** − *RAC875_c53629_483*	83.3	4.6	9	-0.03

Table 5 Genome regions underlying the response to nitrogen (N) for grain yield-BLUPs, adjoining markers (closest in bold), peak position (cM), logarithm of odd (LOD), R^2 (as %) and additive allele in trials at various Australian sites

Chr.	QTL	N effect	Site and year	Adjoining markers	Position (cM)	LOD	R^2 (%)	Allele effect
1A	1	N150-N75	PIN 12	*RAC875_rep_c104986_200* − ***RAC875_c11899_366***	45.7	4.9	8	0.01
		N150-N0	PIN 12	*Ex_c4051_1826* − ***wsnp_Ra_c4664_8410628***	53.9	5.3	9	0.02
1B	2	N150-N0	YAN 11	*Kukri_c16382_396* − ***RAC875_c6789_838***	111.4	3.6	7	0.01
2A	3	N150-N75	PIN 11	*Ra_c18597_329* − ***BS00011893_51***	17.7	4.3	9	-0.03
		N75-N0	YAN 11	***BS00011893_51*** − *Kukri_c46040_620*	19.7	4.9	10	-0.01
		N150-N0	PIN 12	*BS00011893_51* − ***Kukri_c46040_620***	24.7	9.6	20	-0.02
		N150-N0	YAN 11	*BS00011893_51* − ***Kukri_c46040_620***	24.7	5.1	11	0.01
		N150-N75	PIN 12	*BS00011893_51* − ***Kukri_c46040_620***	26.7	11.8	20	-0.02
2B	4	N150-N0	PIN 12	***RFL_Contig3915_1042*** − *wsnp_RFL_Contig4402_5154408*	70	3.2	5	-0.01
3B	5	N87-N52	LAM 12	*Kukri_c32803_84* − ***wPt.7984***	4.9	4	9	0.02
3D2	6	N150-N75	PIN 12	*cfd0064* − ***Excalibur_c3510_1888***	18.7	4.4	7	-0.01
	7	N35-N0	WH 13	*RAC875_rep_c79167_809* − ***CAP12_c1384_314***	79.7	3.7	10	-0.01
5A	8	N75-N0	PIN 12	***BS00028356_51*** − *BS00022646_51*	154.1	3	7	-0.02
6A	9	N35-N0	WH 13	*wsnp_Ex_c2389_4479352* − ***barc0353b***	69.5	4.2	10	0.01
		N60-N0	ED 13	*wsnp_Ex_c2389_4479352* − ***barc0353b***	70.2	4.1	10	-0.02
6B	10	N150-N75	PIN 12	***Ex_c20409_854*** − *Ku_c2392_1692*	38.2	3.3	5	0.01
7A2	11	N75-N0	PIN 11	***BS00068055_51*** − *BobWhite_c23287_57*	0	3.4	8	0.02
		N150-N75	PIN 11	***BS00068055_51*** − *BobWhite_c23287_57*	0	3.4	7	0.02
7B	12	N150-N0	YAN 11	*wPt.9887* − ***BobWhite_c25215_457***	7.1	7.1	14	0.02
		N75-N0	YAN 11	*BobWhite_c25215_457* − ***wsnp_Ra_c3450_6434387***	7.6	5.7	11	-0.01
		N150-N75	PIN 12	*CAP12_c1816_325* − ***Kukri_c109962_396***	13.4	5.1	8	-0.01
		N150-N0	PIN 12	*CAP12_c1816_325* − ***Kukri_c109962_396***	13.4	4.7	8	-0.02

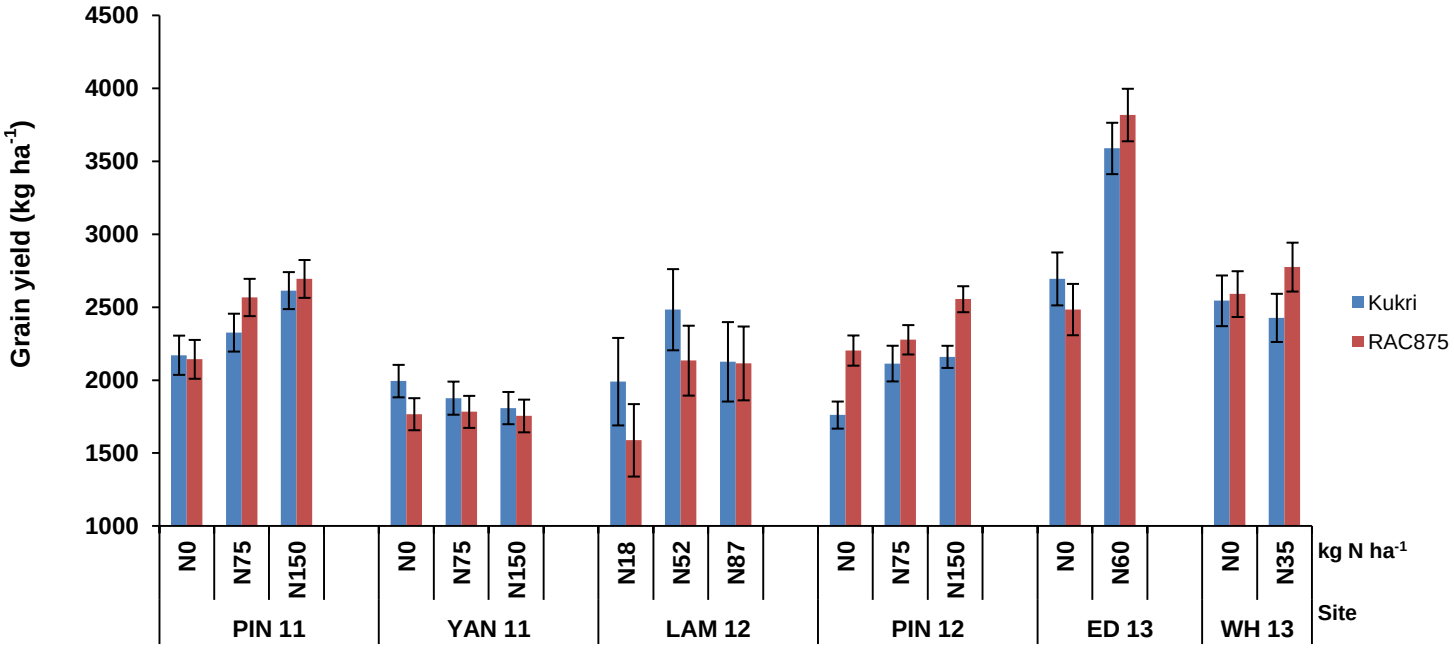


Fig. 1 The performance of parental lines for grain yield (kg ha^{-1}) in six nitrogen (N) use efficiency field trials of Australia. The vertical error bars represent the standard errors of the predicted means after spatial analysis

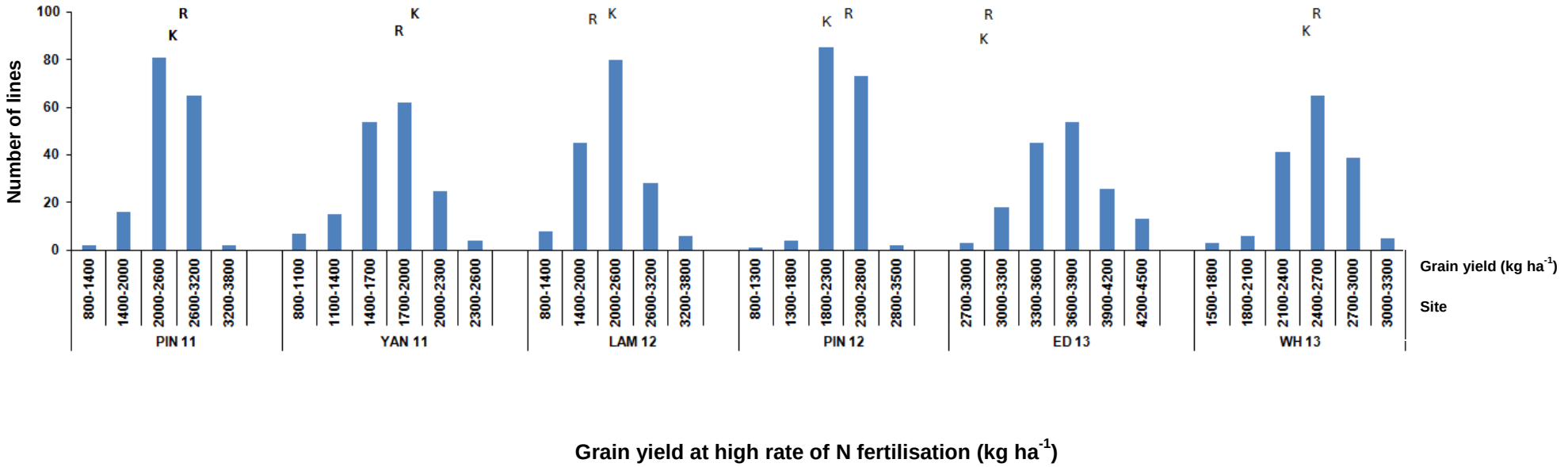


Fig. 2 Distribution of doubled haploid lines for grain yield at high rate of nitrogen (N) fertilisation at all sites

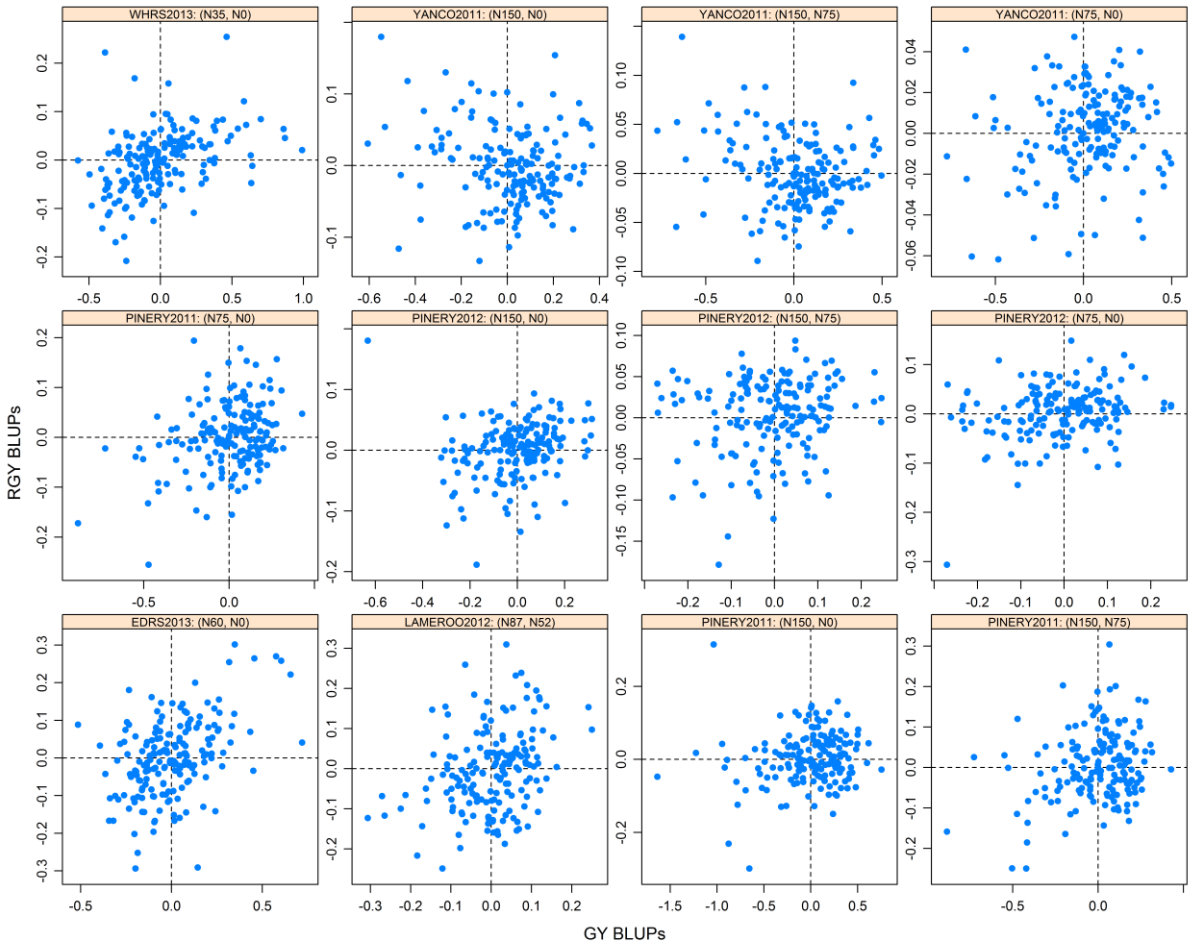


Fig. 3 Responsive grain yield **(**RGY) against grain yield best linear unbiased predictions (GY BLUPs) of individual lines in the population of RAC875 and Kukri across nitrogen use efficiency field trials in Australia

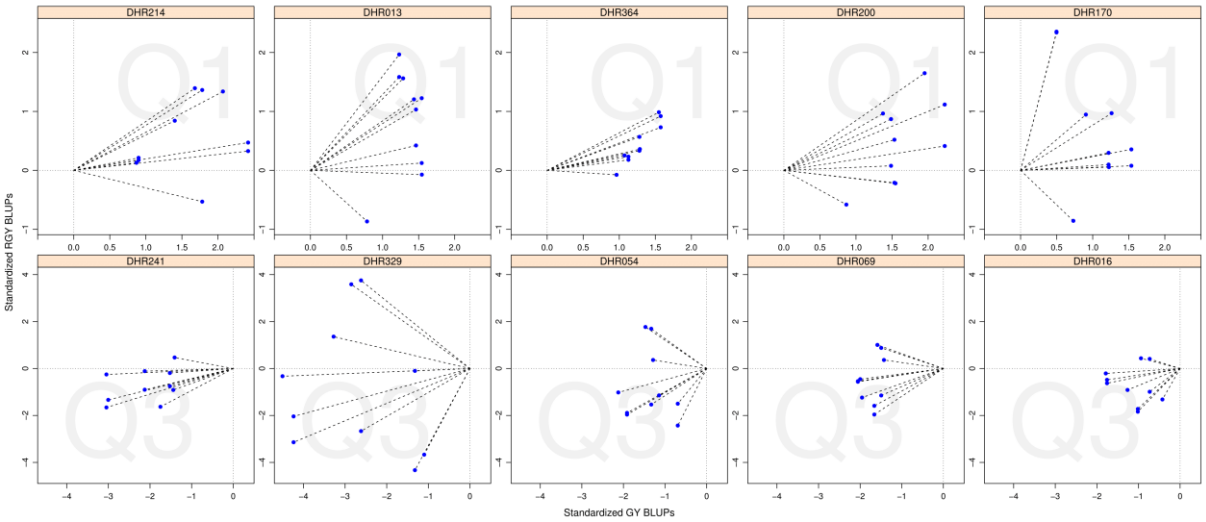


Fig. 4 Top five (upper row of panels) and bottom five (lower row of panels) ranked varieties based on a two-dimensional ranking scheme of the grain yield (GY) and responsive grain yield best linear unbiased **(**RGY BLUPs) for all two level nitrogen treatment combination across the South Australian environments

Supplementary materials Chapter 4

Table 1 Heritability analysis of the sites for grain yield (GY, kg ha^{-1}) at varying nitrogen (N) treatments

Site	N treatment (kg ha^{-1})	Heritability
LAM 12	18	0.00
LAM 12	52	0.18
LAM 12	87	0.26
PIN 11	0	0.75
PIN 11	75	0.90
PIN 11	150	0.87
PIN 12	0	0.56
PIN 12	75	0.71
PIN 12	150	0.56
YAN 11	0	0.50
YAN 11	75	0.33
YAN 11	150	0.39
ED 13	0	0.71
ED 13	60	0.78
WH 13	0	0.73
WH 13	35	0.78

Table 2 Genome regions underlying the single effect of nitrogen (N) on heading date (HD), relative anthesis (RA) and relative maturity (RM), adjoining markers, peak position (cM), logarithm of odd (LOD), R^2 (as, %) and additive allele in various Australian sites

Chr.	Trait	N effect	Site and year	Adjoining markers	Position (cM)	LOD	R^2 (%)	Allele effect
2A	RM	N87	LAM 12	*BobWhite_c1049_338* − ***wsnp_Ex_rep_c69799_68760822***	87.2	4.4	10	0.86
2B	RA	N75	PIN 12	***Tdurum_contig54634_956*** − *TA001874.1495*	2.3	5.3	8	0.92
	RM	N52	LAM 12	*wsnp_JD_c23434_20022750* − ***RAC875_c22997_534***	13.7	4.5	9	0.74
2B	HD	N150	YAN 11	*CAP12_c3807_144* − ***Kukri_c26288_419***	21.7	8.7	12	-1.48
	HD	N75	YAN 11	*CAP12_c3807_144* − ***Kukri_c26288_419***	22.7	6.8	9	-1.28
	HD	N0	YAN 11	*CAP12_c3807_144* − ***Kukri_c26288_419***	23.4	7.5	11	-1.48
	RM	N18	LAM 12	*CAP12_c3807_144* − ***Kukri_c26288_419***	23.4	4	8	0.57
2D	RM	N52	LAM 12	*tplb0057n10_689* − ***RAC875_c24201_984***	35.5	10.2	23	-1.27
	HD	N0	YAN 11	*tplb0057n10_689* − ***RAC875_c24201_984***	36.1	16.1	28	2.41
	RA	N0	PIN 12	*tplb0057n10_689* − ***RAC875_c24201_984***	36.1	5.8	13.2	-1.08
	RA	N75	PIN 12	*tplb0057n10_689* − ***RAC875_c24201_984***	36.1	18.2	36	-2.02
	RM	N87	LAM 12	*tplb0057n10_689* − ***RAC875_c24201_984***	36.1	5.6	12	-1.09
	HD	N75	YAN 11	*tplb0057n10_689* − ***RAC875_c24201_984***	37.1	26.5	52	3.06
	HD	N150	YAN 11	*tplb0057n10_689* − ***RAC875_c24201_984***	37.1	28.2	52	3.19
	RA	N150	PIN 12	*tplb0057n10_689* − ***RAC875_c24201_984***	37.1	18.5	37	-2.14
	RM	N18	LAM 12	*tplb0057n10_689* − ***RAC875_c24201_984***	38.1	9.2	21	-0.96
5B	RM	N52	LAM 12	*RAC875_c2260_1274* − ***Ex_c8501_1020***	195.7	4	8	-0.64
6A	RM	N18	LAM 12	***wsnp_Ex_c2389_4479352*** − *barc0353b*	59.5	3.7	10	-0.56
7A1	RA	N0	PIN 12	*Ku_c12886_1250* − ***Excalibur_c15260_94***	47.5	3.7	9.8	0.84
	HD	N150	YAN 11	*Ku_c12886_1250* − ***Excalibur_c15260_94***	52.4	5.3	7	-1.01
	HD	N75	YAN 11	*Ku_c12886_1250* − ***Excalibur_c15260_94***	52.8	7	9	-1.12
	HD	N0	YAN 11	*BS00011072_51* − ***wsnp_Ku_c6065_10682531***	71	3.7	5	-0.89
7B	HD	N75	YAN 11	***IACX198*** − *BS00081132_51*	0	3.4	4	-0.74
	HD	N0	YAN 11	***IACX198*** − *BS00081132_51*	2	6.5	10	-1.25

Table 3 Genome regions underlying the response to nitrogen (N) for on heading date (HD), relative anthesis (RA) and relative maturity (RM), adjoining markers, peak position (cM), logarithm of odd (LOD), R^2 (as, %) and additive allele in various Australian sites

Chr.	Trait	N effect	Site and year	Adjoining markers	Position (cM)	LOD	R^2 (%)	Allele effect
1A	HD	N52-N0	LAM 12	*wsnp_Ku_c34659_43981982* − ***gdm0128***	36.4	4.5	11	-0.81
	HD	N150-N75	YAN 11	*Excalibur_rep_c110054_341* − ***Excalibur_c8599_133***	100.3	6.3	15	-0.82
	RA	N150-N0	PIN 12	*Tdurum_contig4885_1870* − ***BobWhite_c12305_959***	118.7	3.5	9	-0.96
2A	RA	N87-N52	LAM 12	***BobWhite_c1049_338*** − *wsnp_Ex_rep_c69799_68760822*	84.3	3.8	9.23	1.64
	RA	N87-N18	LAM 12	***BobWhite_c1049_338*** − *wsnp_Ex_rep_c69799_68760822*	84.3	3.6	8.6	0.76
2D	RA	N150-N0	PIN 12	*tplb0057n10_689* − ***RAC875_c24201_984***	39.1	4.5	10	-1.14
	RA	N75-N0	PIN 12	*wsnp_CAP12_c1503_764765* − ***Ex_c10377_845***	55	5.8	14	-1.17
4A	RM	N150-N75	PIN 12	*BS00022839_51* − ***Ex_c66324_1151***	65.4	4.2	10	0.75
4B	RM	N75-N0	PIN 12	***Kukri_c26488_139*** − *Excalibur_c64418_447*	19.2	3.6	8	-1.25

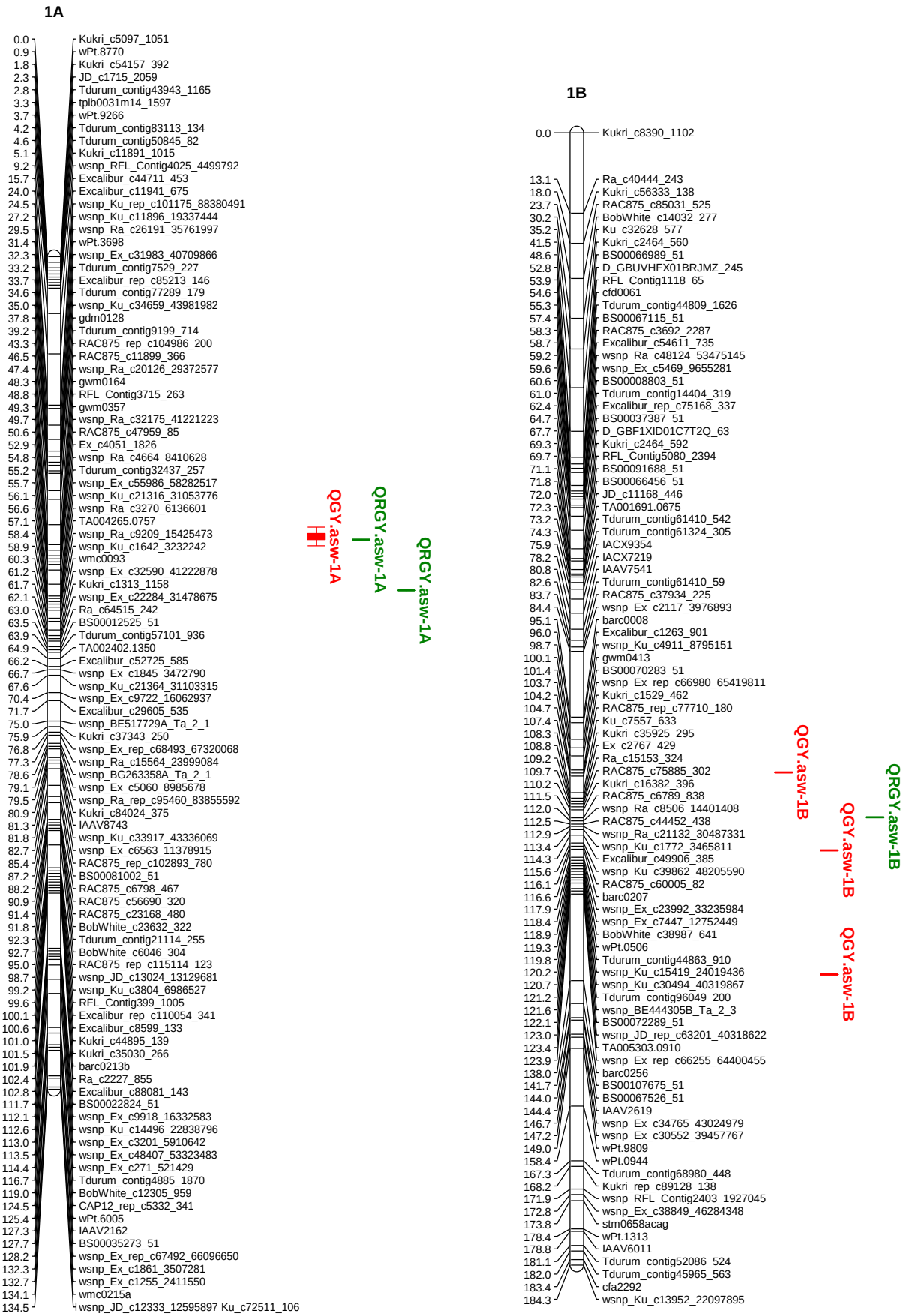
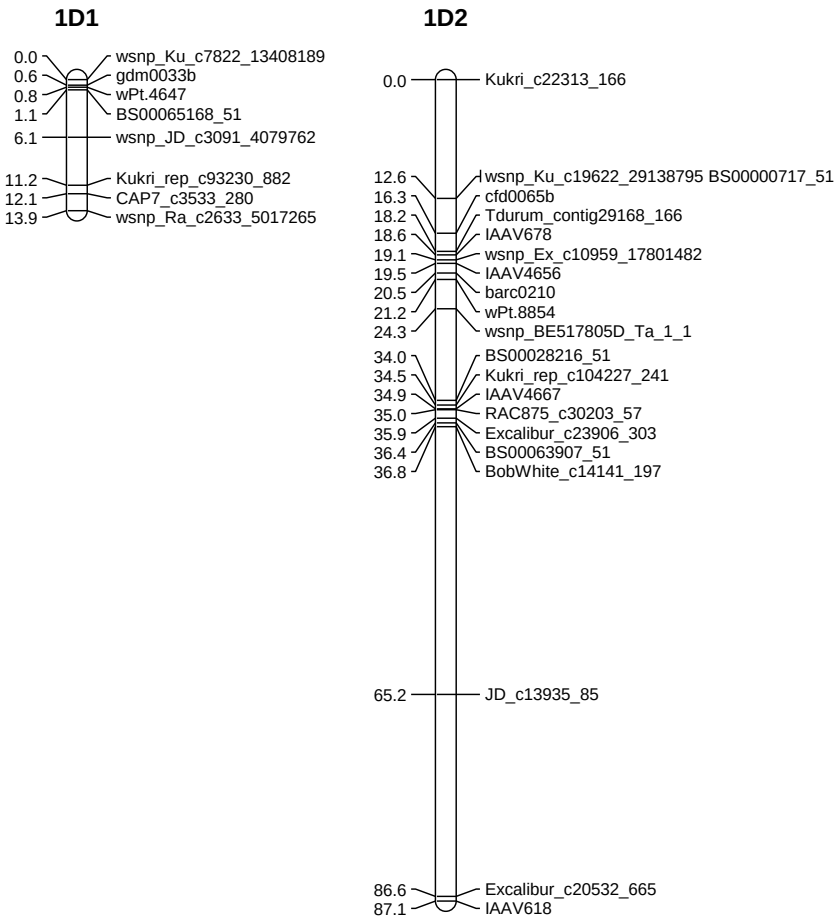
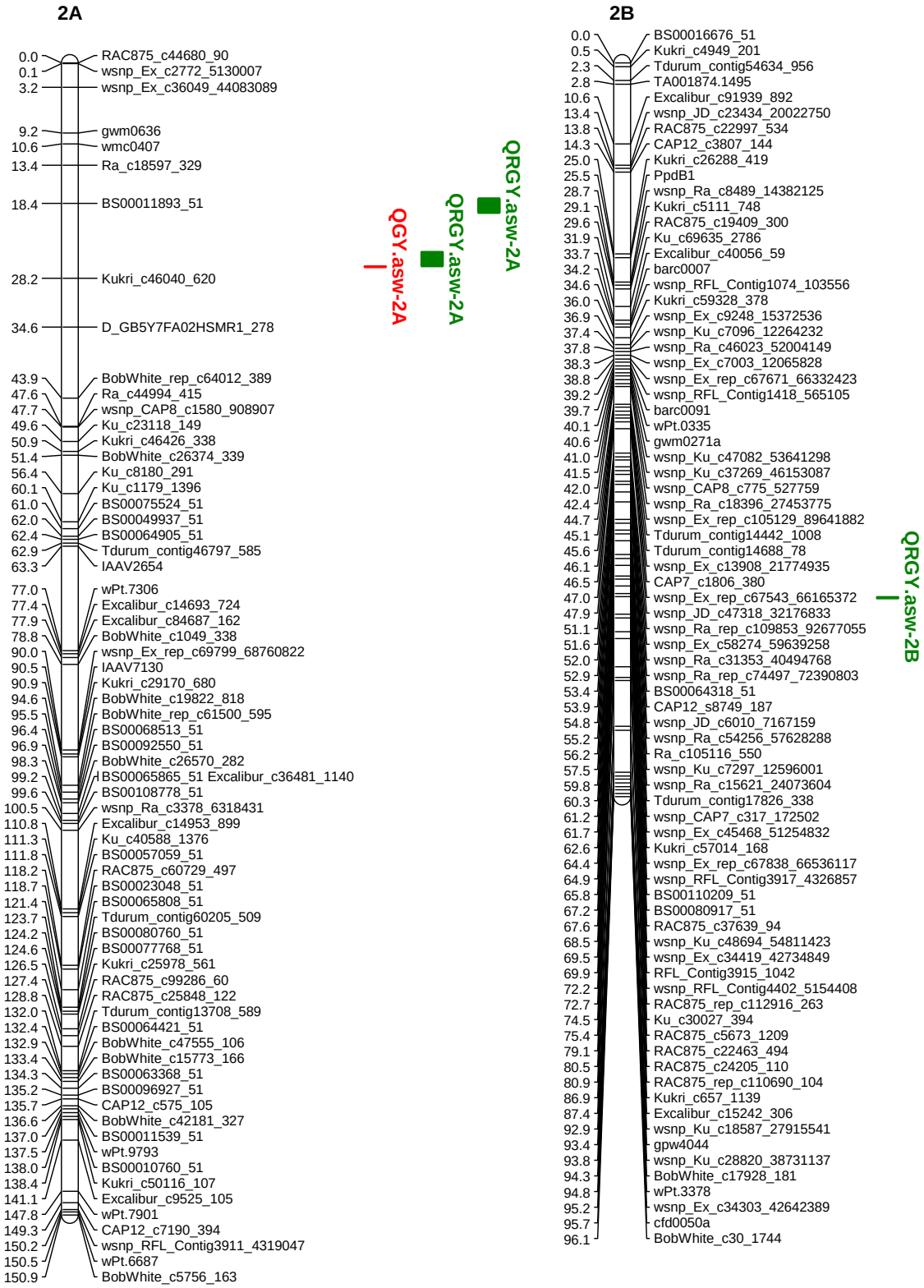
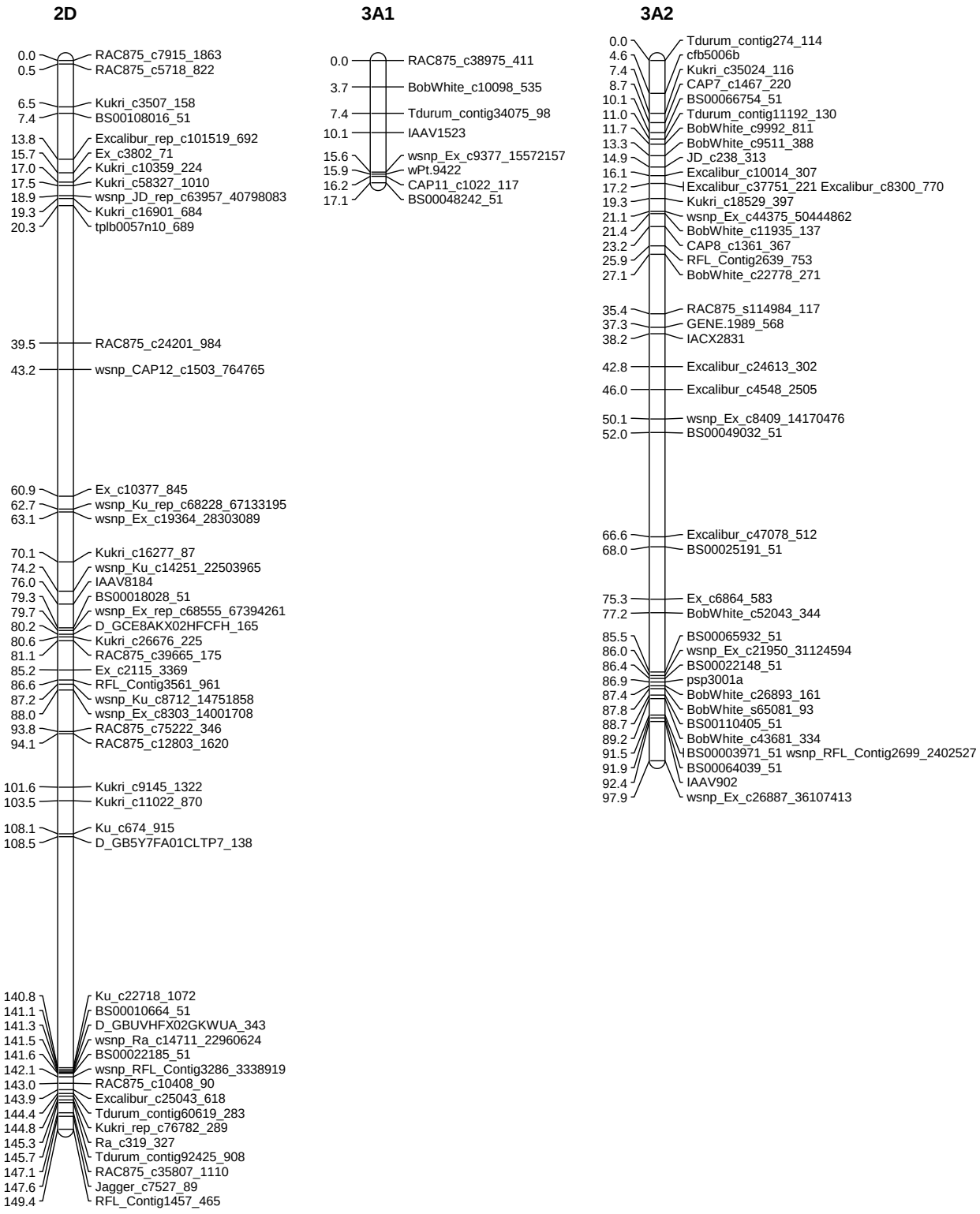
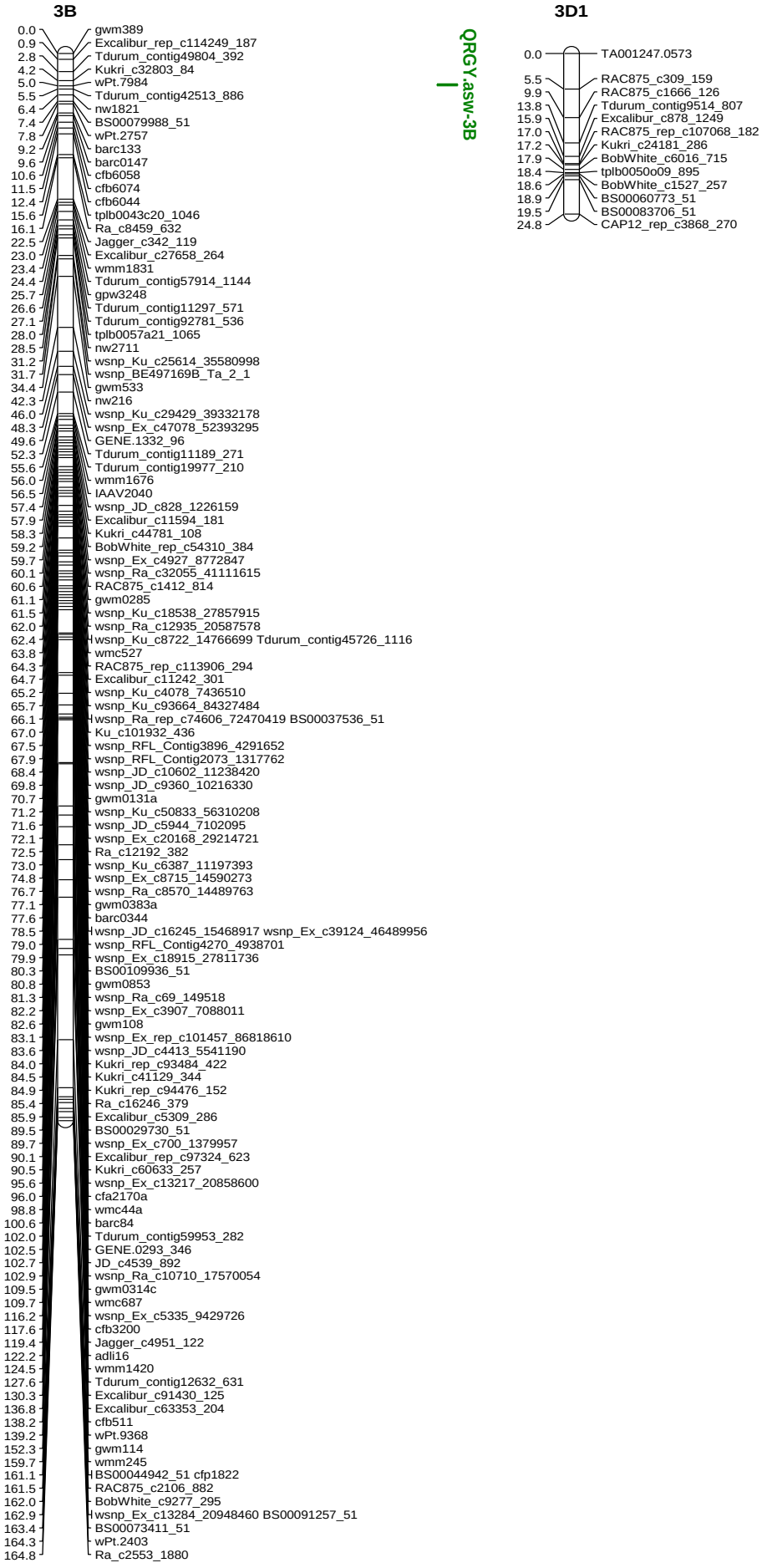


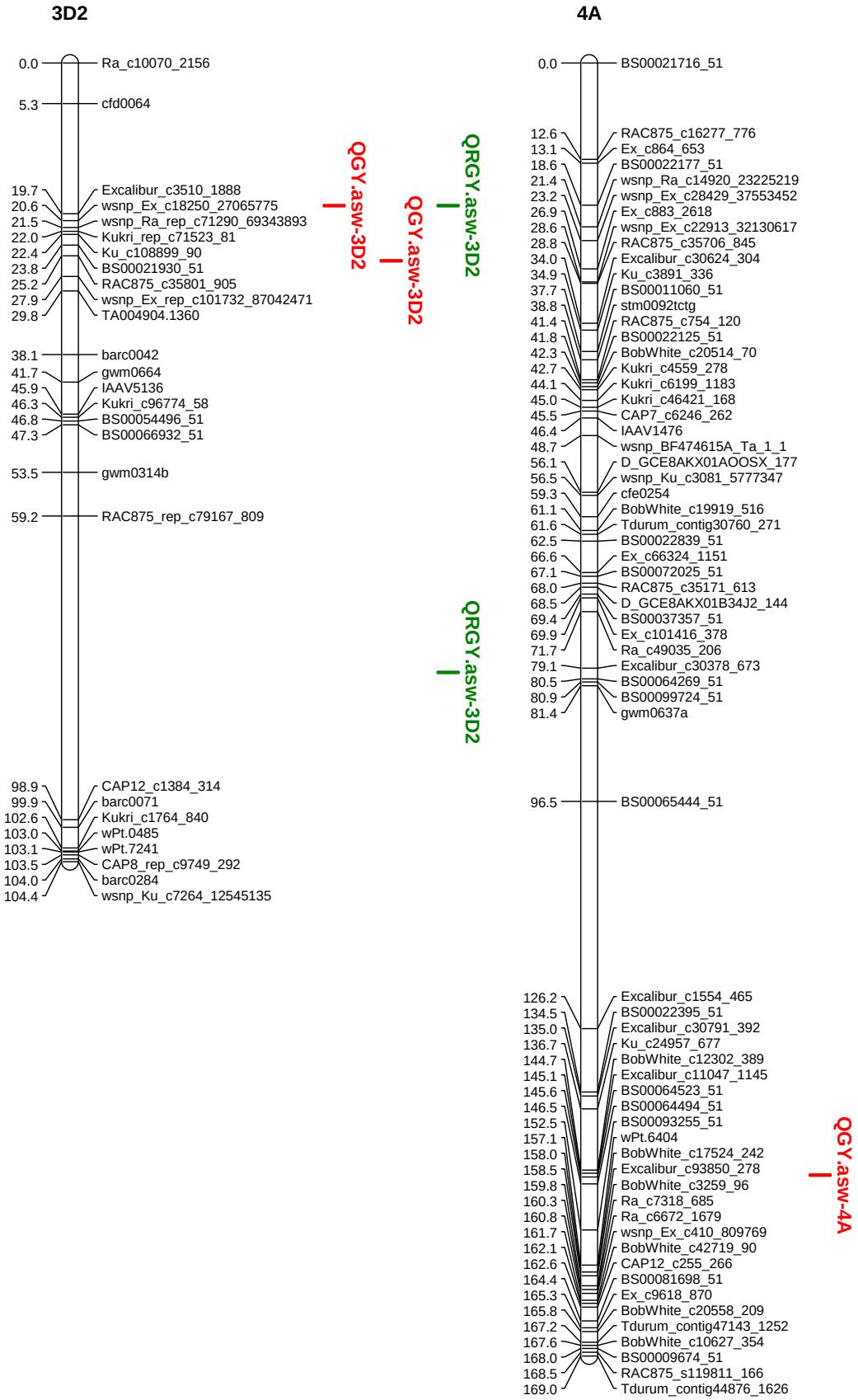
Fig. 1 Significant QTL and markers for grain yield (GY) and response to nitrogen level for GY (RGY). Distances are in cM

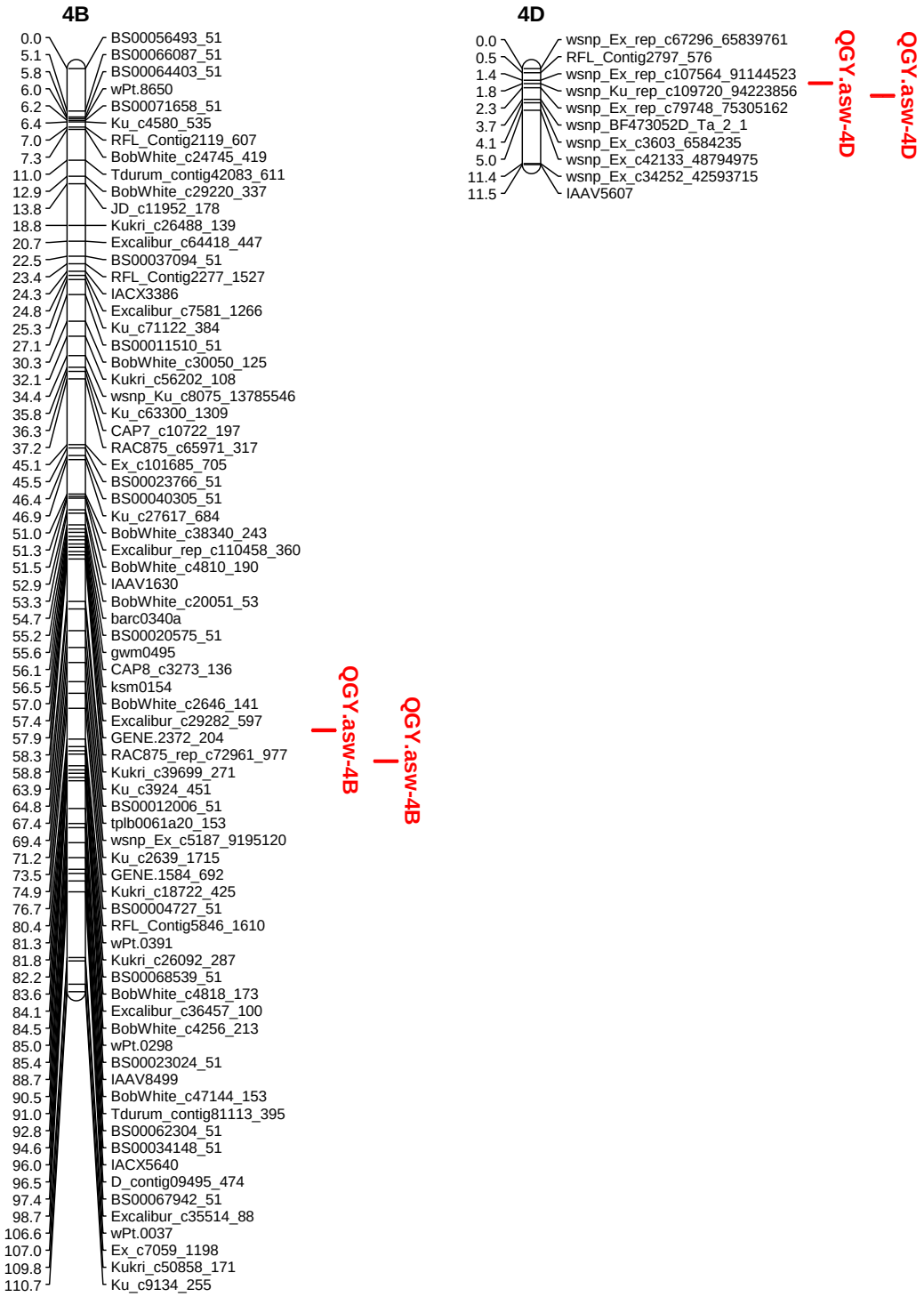

1D1
0.0 wsnp_Ku_c7822_13408189
0.6 gdm0033b
0.8 wPt.4647
1.1 BS00065168_51
6.1 wsnp_JD_c3091_4079762
11.2 Kukri_rep_c93230_882
12.1 CAP7_c3533_280
13.9 wsnp_Ra_c2633_5017265
1D2
0.0 Kukri_c22313_166
12.6 wsnp_Ku_c19622_29138795 BS00000717_51
16.3 cfd0065b
18.2 Tdurum_contig29168_166
18.6 IAAV678
19.1 wsnp_Ex_c10959_17801482
19.5 IAAV4656
20.5 barc0210
21.2 wPt.8854
24.3 wsnp_BE517805D_Ta_1_1
34.0 BS00028216_51
34.5 Kukri_rep_c104227_241
34.9 IAAV4667
35.0 RAC875_c30203_57
35.9 Excalibur_c23906_303
36.4 BS00063907_51
36.8 BobWhite_c14141_197
65.2 JD_c13935_85
86.6 Excalibur_c20532_665
87.1 IAAV618

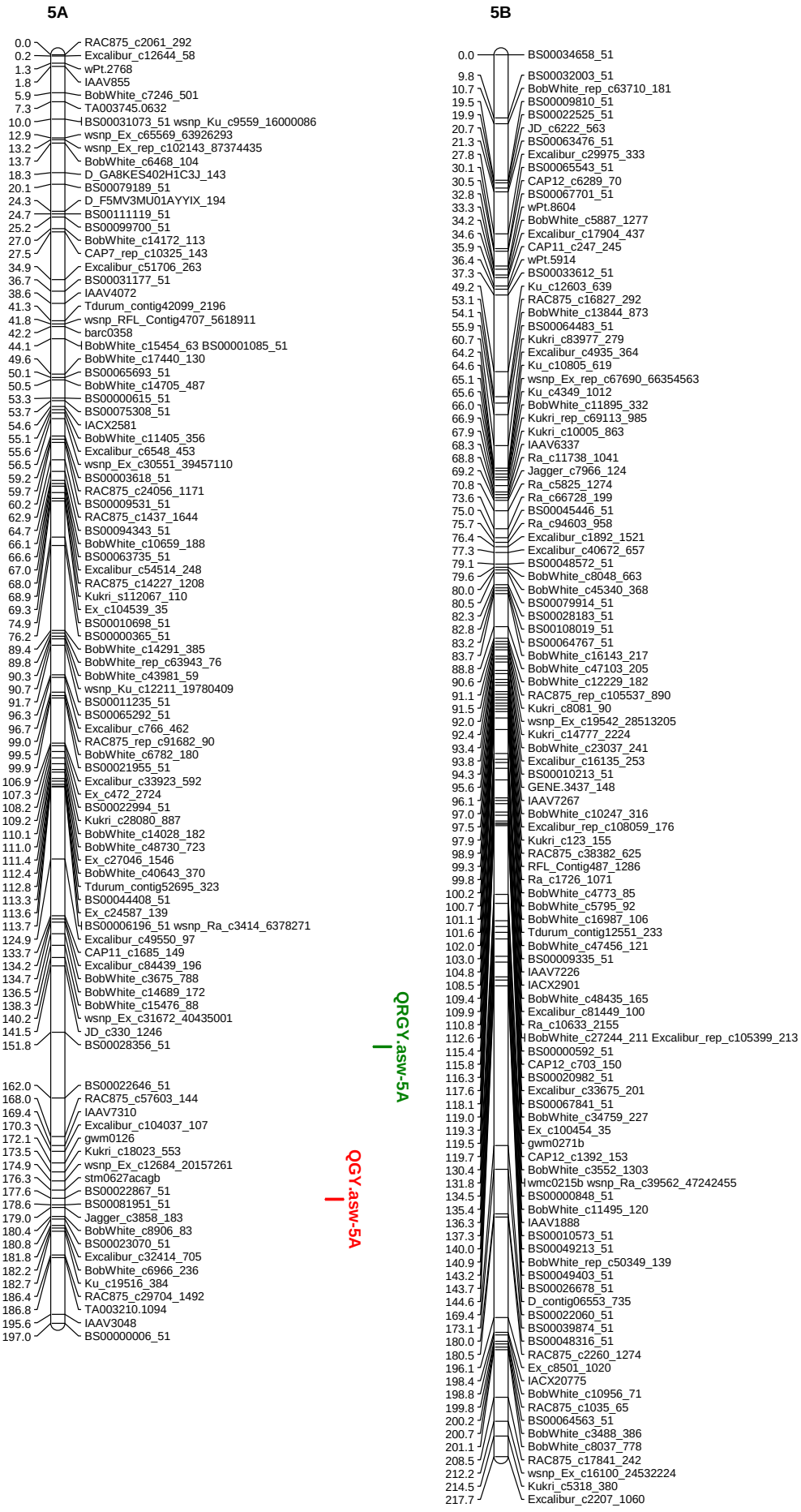

2A
0.0 RAC875_c44680_90
0.1 wsnp_Ex_c2772_5130007
3.2 wsnp_Ex_c36049_44083089
9.2 gwm0636
10.6 wmc0407
13.4 Ra_c18597_329
18.4 BS00011893_51
28.2 Kukri_c46040_620
34.6 D_GB5Y7FA02HSMR1_278
43.9 BobWhite_rep_c64012_389
47.6 Ra_c44994_415
47.7 wsnp_CAP8_c1580_908907
49.6 Ku_c23118_149
50.9 Kukri_c46426_338
51.4 BobWhite_c26374_339
56.4 Ku_c8180_291
60.1 Ku_c1179_1396
61.0 BS00075524_51
62.0 BS00049937_51
62.4 BS00064905_51
62.9 Tdurum_contig46797_585
63.3 IAAV2654
77.0 wPt.7306
77.4 Excalibur_c14693_724
77.9 Excalibur_c84687_162
78.8 BobWhite_c1049_338
90.0 wsnp_Ex_rep_c69799_68760822
90.5 IAAV7130
90.9 Kukri_c29170_680
94.6 BobWhite_c19822_818
95.5 BobWhite_rep_c61500_595
96.4 BS00068513_51
96.9 BS00092550_51
98.3 BobWhite_c26570_282
99.2 BS00065865_51 Excalibur_c36481_1140
99.6 BS00108778_51
100.5 wsnp_Ra_c3378_6318431
110.8 Excalibur_c14953_899
111.3 Ku_c40588_1376
111.8 BS00057059_51
118.2 RAC875_c60729_497
118.7 BS00023048_51
121.4 BS00065808_51
123.7 Tdurum_contig60205_509
124.2 BS00080760_51
124.6 BS00077768_51
126.5 Kukri_c25978_561
127.4 RAC875_c99286_60
128.8 RAC875_c25848_122
132.0 Tdurum_contig13708_589
132.4 BS00064421_51
132.9 BobWhite_c47555_106
133.4 BobWhite_c15773_166
134.3 BS00063368_51
135.2 BS00096927_51
135.7 CAP12_c575_105
136.6 BobWhite_c42181_327
137.0 BS00011539_51
137.5 wPt.9793
138.0 BS00010760_51
138.4 Kukri_c50116_107
141.1 Excalibur_c9525_105
147.8 wPt.7901
149.3 CAP12_c7190_394
150.2 wsnp_RFL_Contig3911_4319047
150.5 wPt.6687
150.9 BobWhite_c5756_163
QGY.asw-2A
QRGY.asw-2A
QRGY.asw-2A
2B
0.0 BS00016676_51
0.5 Kukri_c4949_201
2.3 Tdurum_contig54634_956
2.8 TA001874.1495
10.6 Excalibur_c91939_892
13.4 wsnp_JD_c23434_20022750
13.8 RAC875_c22997_534
14.3 CAP12_c3807_144
25.0 Kukri_c26288_419
25.5 PpdB1
28.7 wsnp_Ra_c8489_14382125
29.1 Kukri_c5111_748
29.6 RAC875_c19409_300
31.9 Ku_c69635_2786
33.7 Excalibur_c40056_59
34.2 barc0007
34.6 wsnp_RFL_Contig1074_103556
36.0 Kukri_c59328_378
36.9 wsnp_Ex_c9248_15372536
37.4 wsnp_Ku_c7096_12264232
37.8 wsnp_Ra_c46023_52004149
38.3 wsnp_Ex_c7003_12065828
38.8 wsnp_Ex_rep_c67671_66332423
39.2 wsnp_RFL_Contig1418_565105
39.7 barc0091
40.1 wPt.0335
40.6 gwm0271a
41.0 wsnp_Ku_c47082_53641298
41.5 wsnp_Ku_c37269_46153087
42.0 wsnp_CAP8_c775_527759
42.4 wsnp_Ra_c18396_27453775
44.7 wsnp_Ex_rep_c105129_89641882
45.1 Tdurum_contig14442_1008
45.6 Tdurum_contig14688_78
46.1 wsnp_Ex_c13908_21774935
46.5 CAP7_c1806_380
47.0 wsnp_Ex_rep_c67543_66165372
47.9 wsnp_JD_c47318_32176833
51.1 wsnp_Ra_rep_c109853_92677055
51.6 wsnp_Ex_c58274_59639258
52.0 wsnp_Ra_c31353_40494768
52.9 wsnp_Ra_rep_c74497_72390803
53.4 BS00064318_51
53.9 CAP12_s8749_187
54.8 wsnp_JD_c6010_7167159
55.2 wsnp_Ra_c54256_57628288
56.2 Ra_c105116_550
57.5 wsnp_Ku_c7297_12596001
59.8 wsnp_Ra_c15621_24073604
60.3 Tdurum_contig17826_338
61.2 wsnp_CAP7_c317_172502
61.7 wsnp_Ex_c45468_51254832
62.6 Kukri_c57014_168
64.4 wsnp_Ex_rep_c67838_66536117
64.9 wsnp_RFL_Contig3917_4326857
65.8 BS00110209_51
67.2 BS00080917_51
67.6 RAC875_c37639_94
68.5 wsnp_Ku_c48694_54811423
69.5 wsnp_Ex_c34419_42734849
69.9 RFL_Contig3915_1042
72.2 wsnp_RFL_Contig4402_5154408
72.7 RAC875_rep_c112916_263
74.5 Ku_c30027_394
75.4 RAC875_c5673_1209
79.1 RAC875_c22463_494
80.5 RAC875_c24205_110
80.9 RAC875_rep_c110690_104
86.9 Kukri_c657_1139
87.4 Excalibur_c15242_306
92.9 wsnp_Ku_c18587_27915541
93.4 gpw4044
93.8 wsnp_Ku_c28820_38731137
94.3 BobWhite_c17928_181
94.8 wPt.3378
95.2 wsnp_Ex_c34303_42642389
95.7 cfd0050a
96.1 BobWhite_c30_1744
QRGY.asw-2B

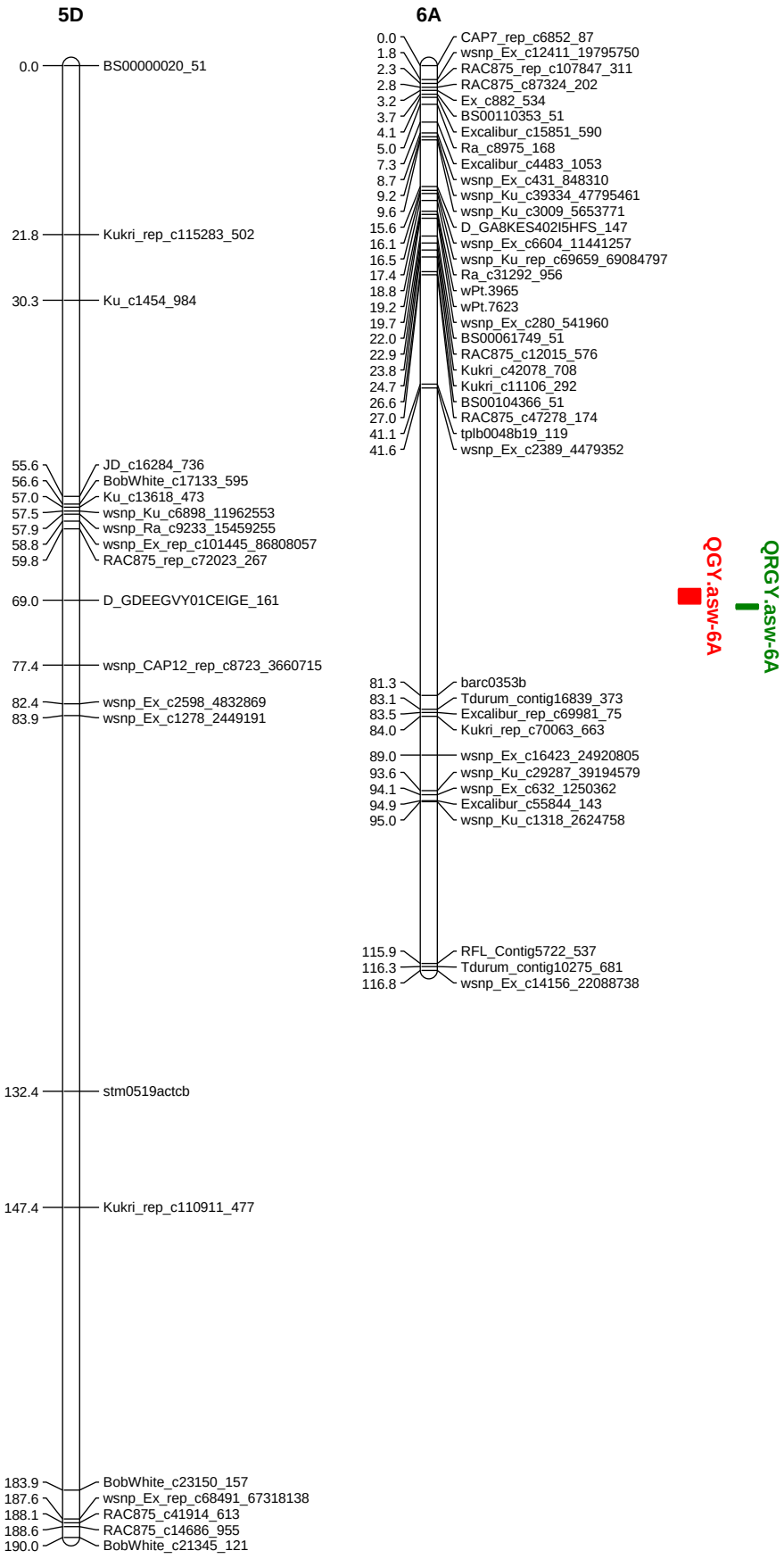

2D
0.0 RAC875_c7915_1863
0.5 RAC875_c5718_822
6.5 Kukri_c3507_158
7.4 BS00108016_51
13.8 Excalibur_rep_c101519_692
15.7 Ex_c3802_71
17.0 Kukri_c10359_224
17.5 Kukri_c58327_1010
18.9 wsnp_JD_rep_c63957_40798083
19.3 Kukri_c16901_684
20.3 tplb0057n10_689
39.5 RAC875_c24201_984
43.2 wsnp_CAP12_c1503_764765
60.9 Ex_c10377_845
62.7 wsnp_Ku_rep_c68228_67133195
63.1 wsnp_Ex_c19364_28303089
70.1 Kukri_c16277_87
74.2 wsnp_Ku_c14251_22503965
76.0 IAAV8184
79.3 BS00018028_51
79.7 wsnp_Ex_rep_c68555_67394261
80.2 D_GCE8AKX02HFCFH_165
80.6 Kukri_c26676_225
81.1 RAC875_c39665_175
85.2 Ex_c2115_3369
86.6 RFL_Contig3561_961
87.2 wsnp_Ku_c8712_14751858
88.0 wsnp_Ex_c8303_14001708
93.8 RAC875_c75222_346
94.1 RAC875_c12803_1620
101.6 Kukri_c9145_1322
103.5 Kukri_c11022_870
108.1 Ku_c674_915
108.5 D_GB5Y7FA01CLTP7_138
140.8 Ku_c22718_1072
141.1 BS00010664_51
141.3 D_GBUVHFX02GKWUA_343
141.5 wsnp_Ra_c14711_22960624
141.6 BS00022185_51
142.1 wsnp_RFL_Contig3286_3338919
143.0 RAC875_c10408_90
143.9 Excalibur_c25043_618
144.4 Tdurum_contig60619_283
144.8 Kukri_rep_c76782_289
145.3 Ra_c319_327
145.7 Tdurum_contig92425_908
147.1 RAC875_c35807_1110
147.6 Jagger_c7527_89
149.4 RFL_Contig1457_465
3A1
0.0 RAC875_c38975_411
3.7 BobWhite_c10098_535
7.4 Tdurum_contig34075_98
10.1 IAAV1523
15.6 wsnp_Ex_c9377_15572157
15.9 wPt.9422
16.2 CAP11_c1022_117
17.1 BS00048242_51
3A2
0.0 Tdurum_contig274_114
4.6 cfb5006b
7.4 Kukri_c35024_116
8.7 CAP7_c1467_220
10.1 BS00066754_51
11.0 Tdurum_contig11192_130
11.7 BobWhite_c9992_811
13.3 BobWhite_c9511_388
14.9 JD_c238_313
16.1 Excalibur_c10014_307
17.2 Excalibur_c37751_221 Excalibur_c8300_770
19.3 Kukri_c18529_397
21.1 wsnp_Ex_c44375_50444862
21.4 BobWhite_c11935_137
23.2 CAP8_c1361_367
25.9 RFL_Contig2639_753
27.1 BobWhite_c22778_271
35.4 RAC875_s114984_117
37.3 GENE.1989_568
38.2 IACX2831
42.8 Excalibur_c24613_302
46.0 Excalibur_c4548_2505
50.1 wsnp_Ex_c8409_14170476
52.0 BS00049032_51
66.6 Excalibur_c47078_512
68.0 BS00025191_51
75.3 Ex_c6864_583
77.2 BobWhite_c52043_344
85.5 BS00065932_51
86.0 wsnp_Ex_c21950_31124594
86.4 BS00022148_51
86.9 psp3001a
87.4 BobWhite_c26893_161
87.8 BobWhite_s65081_93
88.7 BS00110405_51
89.2 BobWhite_c43681_334
91.5 BS00003971_51 wsnp_RFL_Contig2699_2402527
91.9 BS00064039_51
92.4 IAAV902
97.9 wsnp_Ex_c26887_36107413

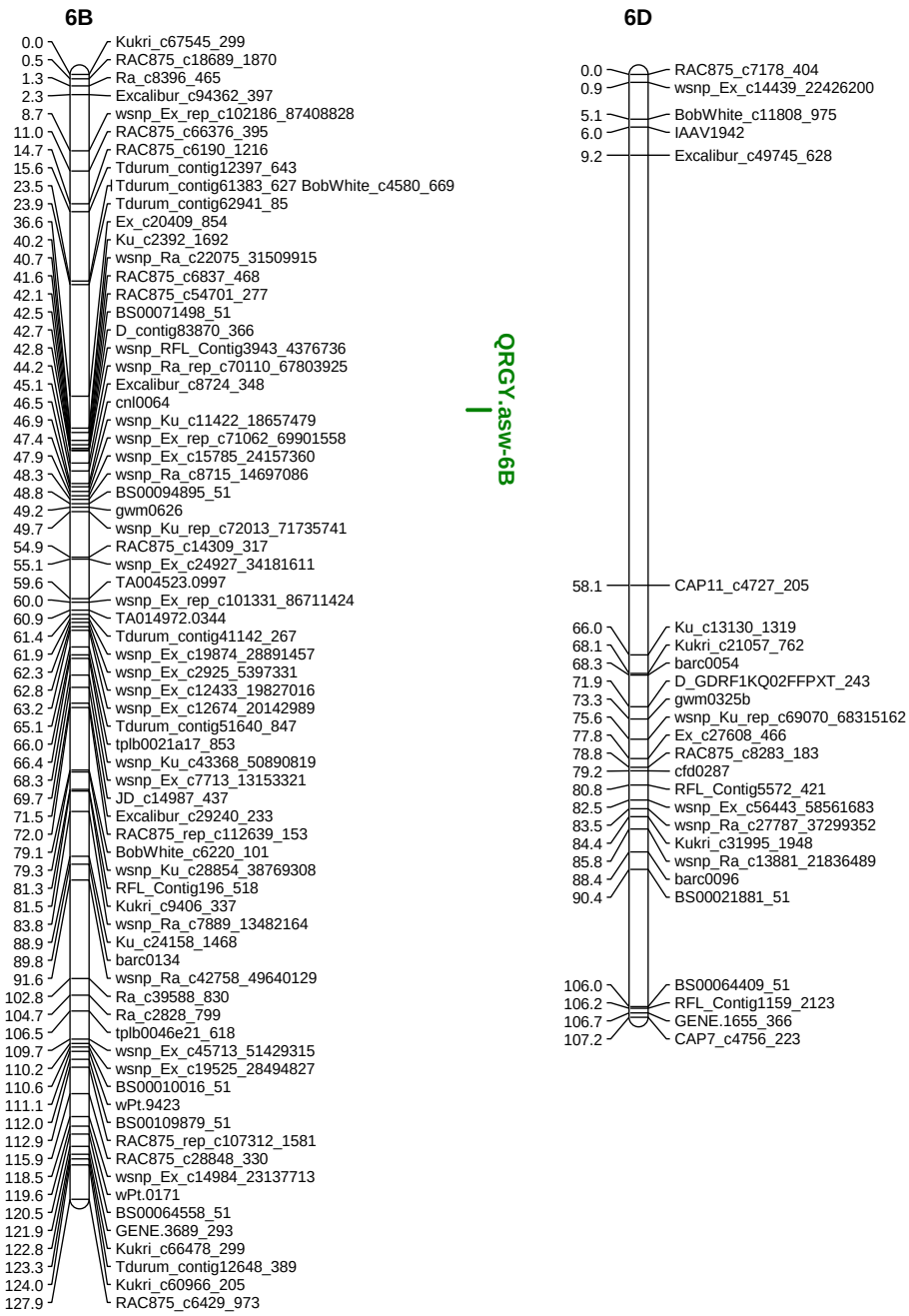

3B
0.0 gwm389
0.9 Excalibur_rep_c114249_187
2.8 Tdurum_contig49804_392
4.2 Kukri_c32803_84
5.0 wPt.7984
5.5 Tdurum_contig42513_886
6.4 nw1821
7.4 BS00079988_51
7.8 wPt.2757
9.2 barc133
9.6 barc0147
10.6 cfb6058
11.5 cfb6074
12.4 cfb6044
15.6 tplb0043c20_1046
16.1 Ra_c8459_632
22.5 Jagger_c342_119
23.0 Excalibur_c27658_264
23.4 wmm1831
24.4 Tdurum_contig57914_1144
25.7 gpw3248
26.6 Tdurum_contig11297_571
27.1 Tdurum_contig92781_536
28.0 tplb0057a21_1065
28.5 nw2711
31.2 wsnp_Ku_c25614_35580998
31.7 wsnp_BE497169B_Ta_2_1
34.4 gwm533
42.3 nw216
46.0 wsnp_Ku_c29429_39332178
48.3 wsnp_Ex_c47078_52393295
49.6 GENE.1332_96
52.3 Tdurum_contig11189_271
55.6 Tdurum_contig19977_210
56.0 wmm1676
56.5 IAAV2040
57.4 wsnp_JD_c828_1226159
57.9 Excalibur_c11594_181
58.3 Kukri_c44781_108
59.2 BobWhite_rep_c54310_384
59.7 wsnp_Ex_c4927_8772847
60.1 wsnp_Ra_c32055_41111615
60.6 RAC875_c1412_814
61.1 gwm0285
61.5 wsnp_Ku_c18538_27857915
62.0 wsnp_Ra_c12935_20587578
62.4 wsnp_Ku_c8722_14766699 Tdurum_contig45726_1116
63.8 wmc527
64.3 RAC875_rep_c113906_294
64.7 Excalibur_c11242_301
65.2 wsnp_Ku_c4078_7436510
65.7 wsnp_Ku_c93664_84327484
66.1 wsnp_Ra_rep_c74606_72470419 BS00037536_51
67.0 Ku_c101932_436
67.5 wsnp_RFL_Contig3896_4291652
67.9 wsnp_RFL_Contig2073_1317762
68.4 wsnp_JD_c10602_11238420
69.8 wsnp_JD_c9360_10216330
70.7 gwm0131a
71.2 wsnp_Ku_c50833_56310208
71.6 wsnp_JD_c5944_7102095
72.1 wsnp_Ex_c20168_29214721
72.5 Ra_c12192_382
73.0 wsnp_Ku_c6387_11197393
74.8 wsnp_Ex_c8715_14590273
76.7 wsnp_Ra_c8570_14489763
77.1 gwm0383a
77.6 barc0344
78.5 wsnp_JD_c16245_15468917 wsnp_Ex_c39124_46489956
79.0 wsnp_RFL_Contig4270_4938701
79.9 wsnp_Ex_c18915_27811736
80.3 BS00109936_51
80.8 gwm0853
81.3 wsnp_Ra_c69_149518
82.2 wsnp_Ex_c3907_7088011
82.6 gwm108
83.1 wsnp_Ex_rep_c101457_86818610
83.6 wsnp_JD_c4413_5541190
84.0 Kukri_rep_c93484_422
84.5 Kukri_c41129_344
84.9 Kukri_rep_c94476_152
85.4 Ra_c16246_379
85.9 Excalibur_c5309_286
89.5 BS00029730_51
89.7 wsnp_Ex_c700_1379957
90.1 Excalibur_rep_c97324_623
90.5 Kukri_c60633_257
95.6 wsnp_Ex_c13217_20858600
96.0 cfa2170a
98.8 wmc44a
100.6 barc84
102.0 Tdurum_contig59953_282
102.5 GENE.0293_346
102.7 JD_c4539_892
102.9 wsnp_Ra_c10710_17570054
109.5 gwm0314c
109.7 wmc687
116.2 wsnp_Ex_c5335_9429726
117.6 cfb3200
119.4 Jagger_c4951_122
122.2 adli16
124.5 wmm1420
127.6 Tdurum_contig12632_631
130.3 Excalibur_c91430_125
136.8 Excalibur_c63353_204
138.2 cfb511
139.2 wPt.9368
152.3 gwm114
159.7 wmm245
161.1 BS00044942_51 cfp1822
161.5 RAC875_c2106_882
162.0 BobWhite_c9277_295
162.9 wsnp_Ex_c13284_20948460 BS00091257_51
163.4 BS00073411_51
164.3 wPt.2403
164.8 Ra_c2553_1880
QRGY.asw-3B
3D1
0.0 TA001247.0573
5.5 RAC875_c309_159
9.9 RAC875_c1666_126
13.8 Tdurum_contig9514_807
15.9 Excalibur_c878_1249
17.0 RAC875_rep_c107068_182
17.2 Kukri_c24181_286
17.9 BobWhite_c6016_715
18.4 tplb0050o09_895
18.6 BobWhite_c1527_257
18.9 BS00060773_51
19.5 BS00083706_51
24.8 CAP12_rep_c3868_270

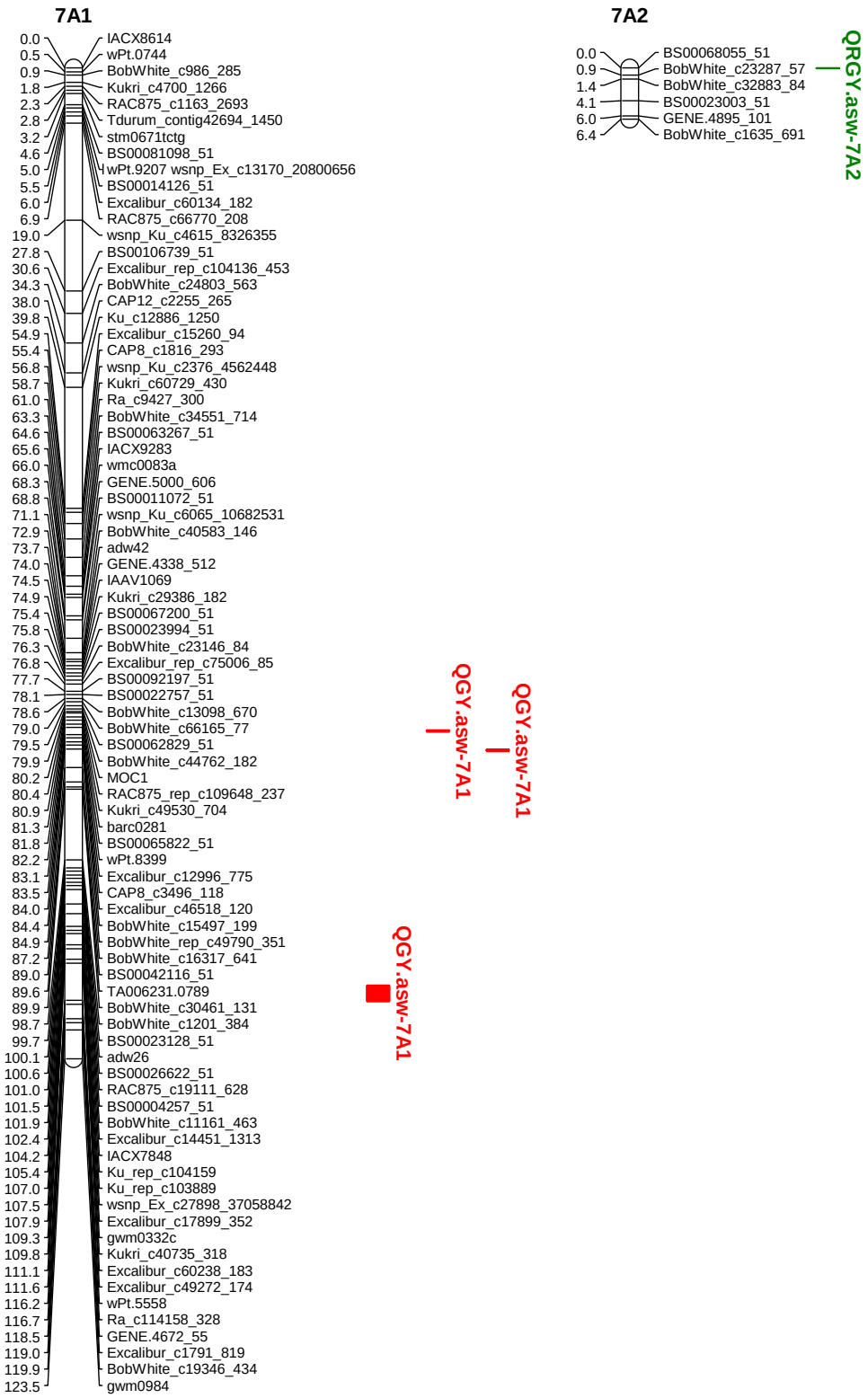

3D2
4A
0.0 Ra_c10070_2156
5.3 cfd0064
19.7 Excalibur_c3510_1888
20.6 wsnp_Ex_c18250_27065775
21.5 wsnp_Ra_rep_c71290_69343893
22.0 Kukri_rep_c71523_81
22.4 Ku_c108899_90
23.8 BS00021930_51
25.2 RAC875_c35801_905
27.9 wsnp_Ex_rep_c101732_87042471
29.8 TA004904.1360
38.1 barc0042
41.7 gwm0664
45.9 IAAV5136
46.3 Kukri_c96774_58
46.8 BS00054496_51
47.3 BS00066932_51
53.5 gwm0314b
59.2 RAC875_rep_c79167_809
98.9 CAP12_c1384_314
99.9 barc0071
102.6 Kukri_c1764_840
103.0 wPt.0485
103.1 wPt.7241
103.5 CAP8_rep_c9749_292
104.0 barc0284
104.4 wsnp_Ku_c7264_12545135
QGY.asw-3D2
QGY.asw-3D2
QRGY.asw-3D2
QRGY.asw-3D2
0.0 BS00021716_51
12.6 RAC875_c16277_776
13.1 Ex_c864_653
18.6 BS00022177_51
21.4 wsnp_Ra_c14920_23225219
23.2 wsnp_Ex_c28429_37553452
26.9 Ex_c883_2618
28.6 wsnp_Ex_c22913_32130617
28.8 RAC875_c35706_845
34.0 Excalibur_c30624_304
34.9 Ku_c3891_336
37.7 BS00011060_51
38.8 stm0092tctg
41.4 RAC875_c754_120
41.8 BS00022125_51
42.3 BobWhite_c20514_70
42.7 Kukri_c4559_278
44.1 Kukri_c6199_1183
45.0 Kukri_c46421_168
45.5 CAP7_c6246_262
46.4 IAAV1476
48.7 wsnp_BF474615A_Ta_1_1
56.1 D_GCE8AKX01AOOSX_177
56.5 wsnp_Ku_c3081_5777347
59.3 cfe0254
61.1 BobWhite_c19919_516
61.6 Tdurum_contig30760_271
62.5 BS00022839_51
66.6 Ex_c66324_1151
67.1 BS00072025_51
68.0 RAC875_c35171_613
68.5 D_GCE8AKX01B34J2_144
69.4 BS00037357_51
69.9 Ex_c101416_378
71.7 Ra_c49035_206
79.1 Excalibur_c30378_673
80.5 BS00064269_51
80.9 BS00099724_51
81.4 gwm0637a
96.5 BS00065444_51
126.2 Excalibur_c1554_465
134.5 BS00022395_51
135.0 Excalibur_c30791_392
136.7 Ku_c24957_677
144.7 BobWhite_c12302_389
145.1 Excalibur_c11047_1145
145.6 BS00064523_51
146.5 BS00064494_51
152.5 BS00093255_51
157.1 wPt.6404
158.0 BobWhite_c17524_242
158.5 Excalibur_c93850_278
159.8 BobWhite_c3259_96
160.3 Ra_c7318_685
160.8 Ra_c6672_1679
161.7 wsnp_Ex_c410_809769
162.1 BobWhite_c42719_90
162.6 CAP12_c255_266
164.4 BS00081698_51
165.3 Ex_c9618_870
165.8 BobWhite_c20558_209
167.2 Tdurum_contig47143_1252
167.6 BobWhite_c10627_354
168.0 BS00009674_51
168.5 RAC875_s119811_166
169.0 Tdurum_contig44876_1626
QGY.asw-4A

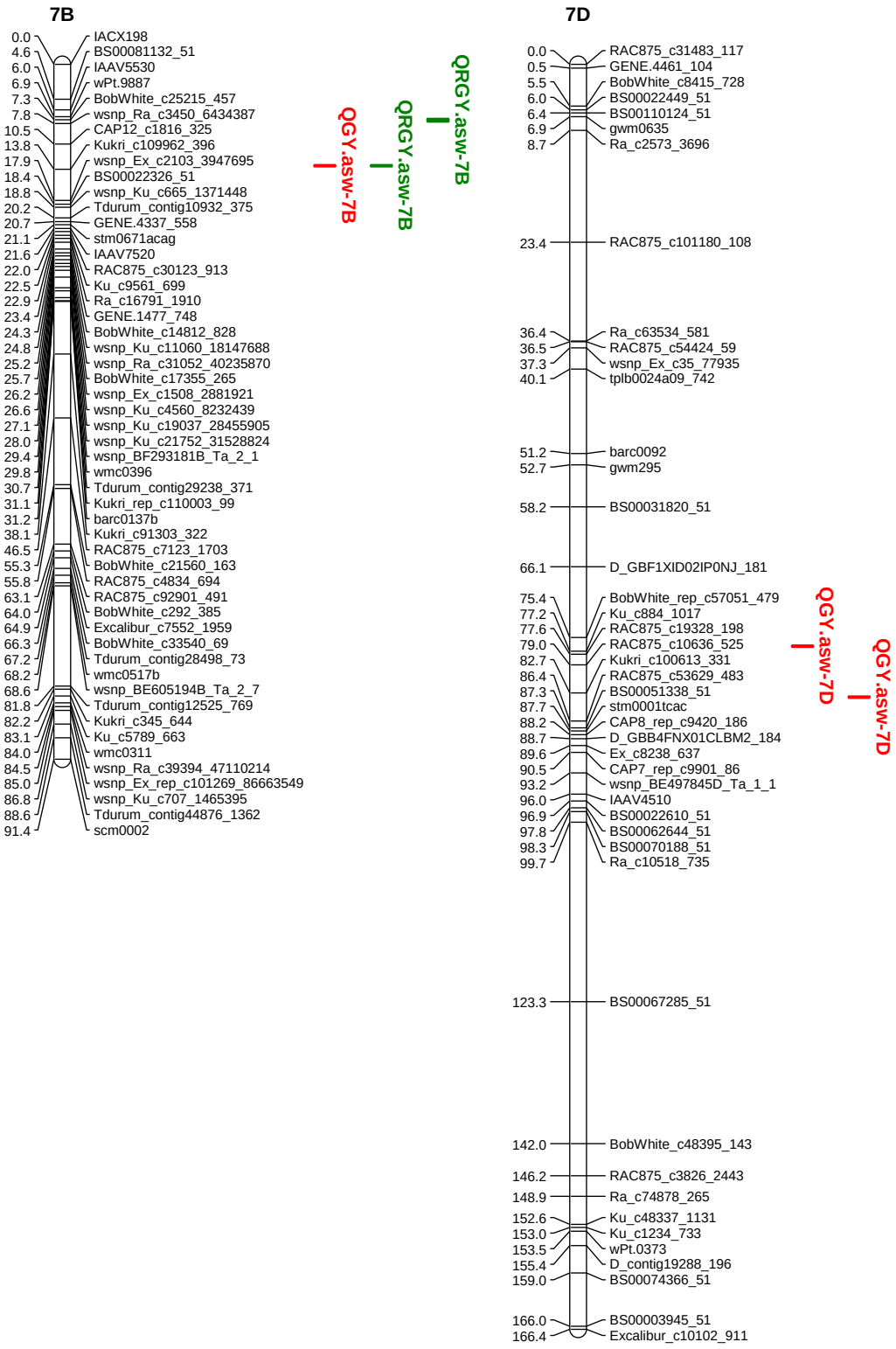

4B
0.0 BS00056493_51
5.1 BS00066087_51
5.8 BS00064403_51
6.0 wPt.8650
6.2 BS00071658_51
6.4 Ku_c4580_535
7.0 RFL_Contig2119_607
7.3 BobWhite_c24745_419
11.0 Tdurum_contig42083_611
12.9 BobWhite_c29220_337
13.8 JD_c11952_178
18.8 Kukri_c26488_139
20.7 Excalibur_c64418_447
22.5 BS00037094_51
23.4 RFL_Contig2277_1527
24.3 IACX3386
24.8 Excalibur_c7581_1266
25.3 Ku_c71122_384
27.1 BS00011510_51
30.3 BobWhite_c30050_125
32.1 Kukri_c56202_108
34.4 wsnp_Ku_c8075_13785546
35.8 Ku_c63300_1309
36.3 CAP7_c10722_197
37.2 RAC875_c65971_317
45.1 Ex_c101685_705
45.5 BS00023766_51
46.4 BS00040305_51
46.9 Ku_c27617_684
51.0 BobWhite_c38340_243
51.3 Excalibur_rep_c110458_360
51.5 BobWhite_c4810_190
52.9 IAAV1630
53.3 BobWhite_c20051_53
54.7 barc0340a
55.2 BS00020575_51
55.6 gwm0495
56.1 CAP8_c3273_136
56.5 ksm0154
57.0 BobWhite_c2646_141
57.4 Excalibur_c29282_597
57.9 GENE.2372_204
58.3 RAC875_rep_c72961_977
58.8 Kukri_c39699_271
63.9 Ku_c3924_451
64.8 BS00012006_51
67.4 tplb0061a20_153
69.4 wsnp_Ex_c5187_9195120
71.2 Ku_c2639_1715
73.5 GENE.1584_692
74.9 Kukri_c18722_425
76.7 BS00004727_51
80.4 RFL_Contig5846_1610
81.3 wPt.0391
81.8 Kukri_c26092_287
82.2 BS00068539_51
83.6 BobWhite_c4818_173
84.1 Excalibur_c36457_100
84.5 BobWhite_c4256_213
85.0 wPt.0298
85.4 BS00023024_51
88.7 IAAV8499
90.5 BobWhite_c47144_153
91.0 Tdurum_contig81113_395
92.8 BS00062304_51
94.6 BS00034148_51
96.0 IACX5640
96.5 D_contig09495_474
97.4 BS00067942_51
98.7 Excalibur_c35514_88
106.6 wPt.0037
107.0 Ex_c7059_1198
109.8 Kukri_c50858_171
110.7 Ku_c9134_255
QGY.asw-4B
QGY.asw-4B
4D
0.0 wsnp_Ex_rep_c67296_65839761
0.5 RFL_Contig2797_576
1.4 wsnp_Ex_rep_c107564_91144523
1.8 wsnp_Ku_rep_c109720_94223856
2.3 wsnp_Ex_rep_c79748_75305162
3.7 wsnp_BF473052D_Ta_2_1
4.1 wsnp_Ex_c3603_6584235
5.0 wsnp_Ex_c42133_48794975
11.4 wsnp_Ex_c34252_42593715
11.5 IAAV5607
QGY.asw-4D
QGY.asw-4D

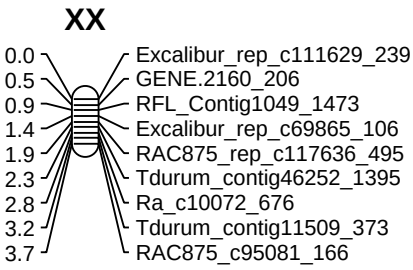

5A
0.0 RAC875_c2061_292
0.2 Excalibur_c12644_58
1.3 wPt.2768
1.8 IAAV855
5.9 BobWhite_c7246_501
7.3 TA003745.0632
10.0 BS00031073_51 wsnp_Ku_c9559_16000086
12.9 wsnp_Ex_c65569_63926293
13.2 wsnp_Ex_rep_c102143_87374435
13.7 BobWhite_c6468_104
18.3 D_GA8KES402H1C3J_143
20.1 BS00079189_51
24.3 D_F5MV3MU01AYYIX_194
24.7 BS00111119_51
25.2 BS00099700_51
27.0 BobWhite_c14172_113
27.5 CAP7_rep_c10325_143
34.9 Excalibur_c51706_263
36.7 BS00031177_51
38.6 IAAV4072
41.3 Tdurum_contig42099_2196
41.8 wsnp_RFL_Contig4707_5618911
42.2 barc0358
44.1 BobWhite_c15454_63 BS00001085_51
49.6 BobWhite_c17440_130
50.1 BS00065693_51
50.5 BobWhite_c14705_487
53.3 BS00000615_51
53.7 BS00075308_51
54.6 IACX2581
55.1 BobWhite_c11405_356
55.6 Excalibur_c6548_453
56.5 wsnp_Ex_c30551_39457110
59.2 BS00003618_51
59.7 RAC875_c24056_1171
60.2 BS00009531_51
62.9 RAC875_c1437_1644
64.7 BS00094343_51
66.1 BobWhite_c10659_188
66.6 BS00063735_51
67.0 Excalibur_c54514_248
68.0 RAC875_c14227_1208
68.9 Kukri_s112067_110
69.3 Ex_c104539_35
74.9 BS00010698_51
76.2 BS00000365_51
89.4 BobWhite_c14291_385
89.8 BobWhite_rep_c63943_76
90.3 BobWhite_c43981_59
90.7 wsnp_Ku_c12211_19780409
91.7 BS00011235_51
96.3 BS00065292_51
96.7 Excalibur_c766_462
99.0 RAC875_rep_c91682_90
99.5 BobWhite_c6782_180
99.9 BS00021955_51
106.9 Excalibur_c33923_592
107.3 Ex_c472_2724
108.2 BS00022994_51
109.2 Kukri_c28080_887
110.1 BobWhite_c14028_182
111.0 BobWhite_c48730_723
111.4 Ex_c27046_1546
112.4 BobWhite_c40643_370
112.8 Tdurum_contig52695_323
113.3 BS00044408_51
113.6 Ex_c24587_139
113.7 BS00006196_51 wsnp_Ra_c3414_6378271
124.9 Excalibur_c49550_97
133.7 CAP11_c1685_149
134.2 Excalibur_c84439_196
134.7 BobWhite_c3675_788
136.5 BobWhite_c14689_172
138.3 BobWhite_c15476_88
140.2 wsnp_Ex_c31672_40435001
141.5 JD_c330_1246
151.8 BS00028356_51
162.0 BS00022646_51
168.0 RAC875_c57603_144
169.4 IAAV7310
170.3 Excalibur_c104037_107
172.1 gwm0126
173.5 Kukri_c18023_553
174.9 wsnp_Ex_c12684_20157261
176.3 stm0627acagb
177.6 BS00022867_51
178.6 BS00081951_51
179.0 Jagger_c3858_183
180.4 BobWhite_c8906_83
180.8 BS00023070_51
181.8 Excalibur_c32414_705
182.2 BobWhite_c6966_236
182.7 Ku_c19516_384
186.4 RAC875_c29704_1492
186.8 TA003210.1094
195.6 IAAV3048
197.0 BS00000006_51
QRGY.asw-5A
QGY.asw-5A
5B
0.0 BS00034658_51
9.8 BS00032003_51
10.7 BobWhite_rep_c63710_181
19.5 BS00009810_51
19.9 BS00022525_51
20.7 JD_c6222_563
21.3 BS00063476_51
27.8 Excalibur_c29975_333
30.1 BS00065543_51
30.5 CAP12_c6289_70
32.8 BS00067701_51
33.3 wPt.8604
34.2 BobWhite_c5887_1277
34.6 Excalibur_c17904_437
35.9 CAP11_c247_245
36.4 wPt.5914
37.3 BS00033612_51
49.2 Ku_c12603_639
53.1 RAC875_c16827_292
54.1 BobWhite_c13844_873
55.9 BS00064483_51
60.7 Kukri_c83977_279
64.2 Excalibur_c4935_364
64.6 Ku_c10805_619
65.1 wsnp_Ex_rep_c67690_66354563
65.6 Ku_c4349_1012
66.0 BobWhite_c11895_332
66.9 Kukri_rep_c69113_985
67.9 Kukri_c10005_863
68.3 IAAV6337
68.8 Ra_c11738_1041
69.2 Jagger_c7966_124
70.8 Ra_c5825_1274
73.6 Ra_c66728_199
75.0 BS00045446_51
75.7 Ra_c94603_958
76.4 Excalibur_c1892_1521
77.3 Excalibur_c40672_657
79.1 BS00048572_51
79.6 BobWhite_c8048_663
80.0 BobWhite_c45340_368
80.5 BS00079914_51
82.3 BS00028183_51
82.8 BS00108019_51
83.2 BS00064767_51
83.7 BobWhite_c16143_217
88.8 BobWhite_c47103_205
90.6 BobWhite_c12229_182
91.1 RAC875_rep_c105537_890
91.5 Kukri_c8081_90
92.0 wsnp_Ex_c19542_28513205
92.4 Kukri_c14777_2224
93.4 BobWhite_c23037_241
93.8 Excalibur_c16135_253
94.3 BS00010213_51
95.6 GENE.3437_148
96.1 IAAV7267
97.0 BobWhite_c10247_316
97.5 Excalibur_rep_c108059_176
97.9 Kukri_c123_155
98.9 RAC875_c38382_625
99.3 RFL_Contig487_1286
99.8 Ra_c1726_1071
100.2 BobWhite_c4773_85
100.7 BobWhite_c5795_92
101.1 BobWhite_c16987_106
101.6 Tdurum_contig12551_233
102.0 BobWhite_c47456_121
103.0 BS00009335_51
104.8 IAAV7226
108.5 IACX2901
109.4 BobWhite_c48435_165
109.9 Excalibur_c81449_100
110.8 Ra_c10633_2155
112.6 BobWhite_c27244_211 Excalibur_rep_c105399_213
115.4 BS00000592_51
115.8 CAP12_c703_150
116.3 BS00020982_51
117.6 Excalibur_c33675_201
118.1 BS00067841_51
119.0 BobWhite_c34759_227
119.3 Ex_c100454_35
119.5 gwm0271b
119.7 CAP12_c1392_153
130.4 BobWhite_c3552_1303
131.8 wmc0215b wsnp_Ra_c39562_47242455
134.5 BS00000848_51
135.4 BobWhite_c11495_120
136.3 IAAV1888
137.3 BS00010573_51
140.0 BS00049213_51
140.9 BobWhite_rep_c50349_139
143.2 BS00049403_51
143.7 BS00026678_51
144.6 D_contig06553_735
169.4 BS00022060_51
173.1 BS00039874_51
180.0 BS00048316_51
180.5 RAC875_c2260_1274
196.1 Ex_c8501_1020
198.4 IACX20775
198.8 BobWhite_c10956_71
199.8 RAC875_c1035_65
200.2 BS00064563_51
200.7 BobWhite_c3488_386
201.1 BobWhite_c8037_778
208.5 RAC875_c17841_242
212.2 wsnp_Ex_c16100_24532224
214.5 Kukri_c5318_380
217.7 Excalibur_c2207_1060


5D
0.0 BS00000020_51
21.8 Kukri_rep_c115283_502
30.3 Ku_c1454_984
55.6 JD_c16284_736
56.6 BobWhite_c17133_595
57.0 Ku_c13618_473
57.5 wsnp_Ku_c6898_11962553
57.9 wsnp_Ra_c9233_15459255
58.8 wsnp_Ex_rep_c101445_86808057
59.8 RAC875_rep_c72023_267
69.0 D_GDEEGVY01CEIGE_161
77.4 wsnp_CAP12_rep_c8723_3660715
82.4 wsnp_Ex_c2598_4832869
83.9 wsnp_Ex_c1278_2449191
132.4 stm0519actcb
147.4 Kukri_rep_c110911_477
183.9 BobWhite_c23150_157
187.6 wsnp_Ex_rep_c68491_67318138
188.1 RAC875_c41914_613
188.6 RAC875_c14686_955
190.0 BobWhite_c21345_121
6A
0.0 CAP7_rep_c6852_87
1.8 wsnp_Ex_c12411_19795750
2.3 RAC875_rep_c107847_311
2.8 RAC875_c87324_202
3.2 Ex_c882_534
3.7 BS00110353_51
4.1 Excalibur_c15851_590
5.0 Ra_c8975_168
7.3 Excalibur_c4483_1053
8.7 wsnp_Ex_c431_848310
9.2 wsnp_Ku_c39334_47795461
9.6 wsnp_Ku_c3009_5653771
15.6 D_GA8KES402I5HFS_147
16.1 wsnp_Ex_c6604_11441257
16.5 wsnp_Ku_rep_c69659_69084797
17.4 Ra_c31292_956
18.8 wPt.3965
19.2 wPt.7623
19.7 wsnp_Ex_c280_541960
22.0 BS00061749_51
22.9 RAC875_c12015_576
23.8 Kukri_c42078_708
24.7 Kukri_c11106_292
26.6 BS00104366_51
27.0 RAC875_c47278_174
41.1 tplb0048b19_119
41.6 wsnp_Ex_c2389_4479352
81.3 barc0353b
83.1 Tdurum_contig16839_373
83.5 Excalibur_rep_c69981_75
84.0 Kukri_rep_c70063_663
89.0 wsnp_Ex_c16423_24920805
93.6 wsnp_Ku_c29287_39194579
94.1 wsnp_Ex_c632_1250362
94.9 Excalibur_c55844_143
95.0 wsnp_Ku_c1318_2624758
115.9 RFL_Contig5722_537
116.3 Tdurum_contig10275_681
116.8 wsnp_Ex_c14156_22088738
QGY.asw-6A
QRGY.asw-6A


6B
0.0 Kukri_c67545_299
0.5 RAC875_c18689_1870
1.3 Ra_c8396_465
2.3 Excalibur_c94362_397
8.7 wsnp_Ex_rep_c102186_87408828
11.0 RAC875_c66376_395
14.7 RAC875_c6190_1216
15.6 Tdurum_contig12397_643
23.5 Tdurum_contig61383_627 BobWhite_c4580_669
23.9 Tdurum_contig62941_85
36.6 Ex_c20409_854
40.2 Ku_c2392_1692
40.7 wsnp_Ra_c22075_31509915
41.6 RAC875_c6837_468
42.1 RAC875_c54701_277
42.5 BS00071498_51
42.7 D_contig83870_366
42.8 wsnp_RFL_Contig3943_4376736
44.2 wsnp_Ra_rep_c70110_67803925
45.1 Excalibur_c8724_348
46.5 cnl0064
46.9 wsnp_Ku_c11422_18657479
47.4 wsnp_Ex_rep_c71062_69901558
47.9 wsnp_Ex_c15785_24157360
48.3 wsnp_Ra_c8715_14697086
48.8 BS00094895_51
49.2 gwm0626
49.7 wsnp_Ku_rep_c72013_71735741
54.9 RAC875_c14309_317
55.1 wsnp_Ex_c24927_34181611
59.6 TA004523.0997
60.0 wsnp_Ex_rep_c101331_86711424
60.9 TA014972.0344
61.4 Tdurum_contig41142_267
61.9 wsnp_Ex_c19874_28891457
62.3 wsnp_Ex_c2925_5397331
62.8 wsnp_Ex_c12433_19827016
63.2 wsnp_Ex_c12674_20142989
65.1 Tdurum_contig51640_847
66.0 tplb0021a17_853
66.4 wsnp_Ku_c43368_50890819
68.3 wsnp_Ex_c7713_13153321
69.7 JD_c14987_437
71.5 Excalibur_c29240_233
72.0 RAC875_rep_c112639_153
79.1 BobWhite_c6220_101
79.3 wsnp_Ku_c28854_38769308
81.3 RFL_Contig196_518
81.5 Kukri_c9406_337
83.8 wsnp_Ra_c7889_13482164
88.9 Ku_c24158_1468
89.8 barc0134
91.6 wsnp_Ra_c42758_49640129
102.8 Ra_c39588_830
104.7 Ra_c2828_799
106.5 tplb0046e21_618
109.7 wsnp_Ex_c45713_51429315
110.2 wsnp_Ex_c19525_28494827
110.6 BS00010016_51
111.1 wPt.9423
112.0 BS00109879_51
112.9 RAC875_rep_c107312_1581
115.9 RAC875_c28848_330
118.5 wsnp_Ex_c14984_23137713
119.6 wPt.0171
120.5 BS00064558_51
121.9 GENE.3689_293
122.8 Kukri_c66478_299
123.3 Tdurum_contig12648_389
124.0 Kukri_c60966_205
127.9 RAC875_c6429_973
QRGY.asw-6B
6D
0.0 RAC875_c7178_404
0.9 wsnp_Ex_c14439_22426200
5.1 BobWhite_c11808_975
6.0 IAAV1942
9.2 Excalibur_c49745_628
58.1 CAP11_c4727_205
66.0 Ku_c13130_1319
68.1 Kukri_c21057_762
68.3 barc0054
71.9 D_GDRF1KQ02FFPXT_243
73.3 gwm0325b
75.6 wsnp_Ku_rep_c69070_68315162
77.8 Ex_c27608_466
78.8 RAC875_c8283_183
79.2 cfd0287
80.8 RFL_Contig5572_421
82.5 wsnp_Ex_c56443_58561683
83.5 wsnp_Ra_c27787_37299352
84.4 Kukri_c31995_1948
85.8 wsnp_Ra_c13881_21836489
88.4 barc0096
90.4 BS00021881_51
106.0 BS00064409_51
106.2 RFL_Contig1159_2123
106.7 GENE.1655_366
107.2 CAP7_c4756_223


7A1
0.0 IACX8614
0.5 wPt.0744
0.9 BobWhite_c986_285
1.8 Kukri_c4700_1266
2.3 RAC875_c1163_2693
2.8 Tdurum_contig42694_1450
3.2 stm0671tctg
4.6 BS00081098_51
5.0 wPt.9207 wsnp_Ex_c13170_20800656
5.5 BS00014126_51
6.0 Excalibur_c60134_182
6.9 RAC875_c66770_208
19.0 wsnp_Ku_c4615_8326355
27.8 BS00106739_51
30.6 Excalibur_rep_c104136_453
34.3 BobWhite_c24803_563
38.0 CAP12_c2255_265
39.8 Ku_c12886_1250
54.9 Excalibur_c15260_94
55.4 CAP8_c1816_293
56.8 wsnp_Ku_c2376_4562448
58.7 Kukri_c60729_430
61.0 Ra_c9427_300
63.3 BobWhite_c34551_714
64.6 BS00063267_51
65.6 IACX9283
66.0 wmc0083a
68.3 GENE.5000_606
68.8 BS00011072_51
71.1 wsnp_Ku_c6065_10682531
72.9 BobWhite_c40583_146
73.7 adw42
74.0 GENE.4338_512
74.5 IAAV1069
74.9 Kukri_c29386_182
75.4 BS00067200_51
75.8 BS00023994_51
76.3 BobWhite_c23146_84
76.8 Excalibur_rep_c75006_85
77.7 BS00092197_51
78.1 BS00022757_51
78.6 BobWhite_c13098_670
79.0 BobWhite_c66165_77
79.5 BS00062829_51
79.9 BobWhite_c44762_182
80.2 MOC1
80.4 RAC875_rep_c109648_237
80.9 Kukri_c49530_704
81.3 barc0281
81.8 BS00065822_51
82.2 wPt.8399
83.1 Excalibur_c12996_775
83.5 CAP8_c3496_118
84.0 Excalibur_c46518_120
84.4 BobWhite_c15497_199
84.9 BobWhite_rep_c49790_351
87.2 BobWhite_c16317_641
89.0 BS00042116_51
89.6 TA006231.0789
89.9 BobWhite_c30461_131
98.7 BobWhite_c1201_384
99.7 BS00023128_51
100.1 adw26
100.6 BS00026622_51
101.0 RAC875_c19111_628
101.5 BS00004257_51
101.9 BobWhite_c11161_463
102.4 Excalibur_c14451_1313
104.2 IACX7848
105.4 Ku_rep_c104159
107.0 Ku_rep_c103889
107.5 wsnp_Ex_c27898_37058842
107.9 Excalibur_c17899_352
109.3 gwm0332c
109.8 Kukri_c40735_318
111.1 Excalibur_c60238_183
111.6 Excalibur_c49272_174
116.2 wPt.5558
116.7 Ra_c114158_328
118.5 GENE.4672_55
119.0 Excalibur_c1791_819
119.9 BobWhite_c19346_434
123.5 gwm0984
QGY.asw-7A1
QGY.asw-7A1
QGY.asw-7A1
7A2
0.0 BS00068055_51
0.9 BobWhite_c23287_57
1.4 BobWhite_c32883_84
4.1 BS00023003_51
6.0 GENE.4895_101
6.4 BobWhite_c1635_691
QRGY.asw-7A2


7B
0.0 IACX198
4.6 BS00081132_51
6.0 IAAV5530
6.9 wPt.9887
7.3 BobWhite_c25215_457
7.8 wsnp_Ra_c3450_6434387
10.5 CAP12_c1816_325
13.8 Kukri_c109962_396
17.9 wsnp_Ex_c2103_3947695
18.4 BS00022326_51
18.8 wsnp_Ku_c665_1371448
20.2 Tdurum_contig10932_375
20.7 GENE.4337_558
21.1 stm0671acag
21.6 IAAV7520
22.0 RAC875_c30123_913
22.5 Ku_c9561_699
22.9 Ra_c16791_1910
23.4 GENE.1477_748
24.3 BobWhite_c14812_828
24.8 wsnp_Ku_c11060_18147688
25.2 wsnp_Ra_c31052_40235870
25.7 BobWhite_c17355_265
26.2 wsnp_Ex_c1508_2881921
26.6 wsnp_Ku_c4560_8232439
27.1 wsnp_Ku_c19037_28455905
28.0 wsnp_Ku_c21752_31528824
29.4 wsnp_BF293181B_Ta_2_1
29.8 wmc0396
30.7 Tdurum_contig29238_371
31.1 Kukri_rep_c110003_99
31.2 barc0137b
38.1 Kukri_c91303_322
46.5 RAC875_c7123_1703
55.3 BobWhite_c21560_163
55.8 RAC875_c4834_694
63.1 RAC875_c92901_491
64.0 BobWhite_c292_385
64.9 Excalibur_c7552_1959
66.3 BobWhite_c33540_69
67.2 Tdurum_contig28498_73
68.2 wmc0517b
68.6 wsnp_BE605194B_Ta_2_7
81.8 Tdurum_contig12525_769
82.2 Kukri_c345_644
83.1 Ku_c5789_663
84.0 wmc0311
84.5 wsnp_Ra_c39394_47110214
85.0 wsnp_Ex_rep_c101269_86663549
86.8 wsnp_Ku_c707_1465395
88.6 Tdurum_contig44876_1362
91.4 scm0002
QGY.asw-7B
QRGY.asw-7B
QRGY.asw-7B
7D
0.0 RAC875_c31483_117
0.5 GENE.4461_104
5.5 BobWhite_c8415_728
6.0 BS00022449_51
6.4 BS00110124_51
6.9 gwm0635
8.7 Ra_c2573_3696
23.4 RAC875_c101180_108
36.4 Ra_c63534_581
36.5 RAC875_c54424_59
37.3 wsnp_Ex_c35_77935
40.1 tplb0024a09_742
51.2 barc0092
52.7 gwm295
58.2 BS00031820_51
66.1 D_GBF1XID02IP0NJ_181
75.4 BobWhite_rep_c57051_479
77.2 Ku_c884_1017
77.6 RAC875_c19328_198
79.0 RAC875_c10636_525
82.7 Kukri_c100613_331
86.4 RAC875_c53629_483
87.3 BS00051338_51
87.7 stm0001tcac
88.2 CAP8_rep_c9420_186
88.7 D_GBB4FNX01CLBM2_184
89.6 Ex_c8238_637
90.5 CAP7_rep_c9901_86
93.2 wsnp_BE497845D_Ta_1_1
96.0 IAAV4510
96.9 BS00022610_51
97.8 BS00062644_51
98.3 BS00070188_51
99.7 Ra_c10518_735
123.3 BS00067285_51
142.0 BobWhite_c48395_143
146.2 RAC875_c3826_2443
148.9 Ra_c74878_265
152.6 Ku_c48337_1131
153.0 Ku_c1234_733
153.5 wPt.0373
155.4 D_contig19288_196
159.0 BS00074366_51
166.0 BS00003945_51
166.4 Excalibur_c10102_911
QGY.asw-7D
QGY.asw-7D


XX
0.0
0.5
0.9
1.4
1.9
2.3
2.8
3.2
3.7
Excalibur_rep_c111629_239
GENE.2160_206
RFL_Contig1049_1473
Excalibur_rep_c69865_106
RAC875_rep_c117636_495
Tdurum_contig46252_1395
Ra_c10072_676
Tdurum_contig11509_373
RAC875_c95081_166

Chapter 5

Statement of Authorship

Title of Paper: The genetic control of grain protein content under variable nitrogen supply in an Australian mapping population

Publication Status: Publication Style

Publication Details: Saba Mahjourimajd, Julian Taylor, Zed Rengel, Hossein Khabaz-Saberi, Haydn Kuchel, Mamoru Okamoto, Peter Langridge, 2015, Euphytica

Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Saba Mahjourimajd Performed analysis on all samples, interpreted data and wrote manuscript

Signature **Date** 17/12/14

Prof. Zed Rengel Provided research data, contributed to research ideas and design

Signature **Date** 04 Dec 2014

Dr. Hossein Khabaz-Saberi Contributed in filed experiments in the Western Australia and measured data

Signature **Date 09/12/2014**

Dr. Haydn Kuchel Supervised development of work, helped in data interpretation and manuscript evaluation

Signature **Date** 15/12/14

Dr. Mamoru Okamoto Supervised development of work, helped in data interpretation and manuscript evaluation

Signature **Date** 15/12/2014

Prof. Peter Langridge Supervised development of work, helped in data interpretation and manuscript evaluation

Signature **Date** 15/12/2014

The genetic control of grain protein content under variable nitrogen supply in an Australian mapping population

Saba Mahjourimajd[1], Julian Taylor [3], Zed Rengel[4], Hossein Khabaz-Saberi[4], Haydn Kuchel[2], Mamoru Okamoto[1], Peter Langridge[1],

[1]Australian Centre for Plant Functional Genomics (ACPFG), University of Adelaide, PMB1, Glen Osmond, SA 5064, Australia

[2]Australian Grain Technologies, PMB1, Glen Osmond, SA 5064, Australia

[3]School of Agriculture, Food and Wine, Waite Research Institute, University of Adelaide, PMB 1, Glen Osmond, SA 5064, Australia

[4]Soil Science and Plant Nutrition M087, School of Earth and Environment, University of Western Australia, 35 Stirling Highway, Crawley WA 6009, Australia

Abstract

Genetic variation has been observed in both protein concentration in wheat grain and total protein content (protein yield). Here we describe the genetic analysis of variation for grain protein in response to nitrogen (N) and locate significant genome regions controlling grain protein components in a spring wheat population. In total, six N use efficiency (NUE) field trials were carried out for the target traits in a sub-population of doubled haploid lines derived from a cross between two Australian varieties, RAC875 and Kukri, in Southern and Western Australia from 2011 to 2013. Twenty four putative Quantitative Trait Loci for the protein-related traits were identified at high and low N supply and ten QTL were identified for the response to N of the traits studied. These loci accounted for a significant proportion of the overall effect of N supply. Several of the regions were co-localised with grain yield QTL and are promising targets for further investigation and selection in breeding programs.

Keywords Nitrogen use efficiency (NUE). Grain protein concentration (GPC). Protein yield (PY). Quantitative trait loci (QTL), wheat, breeding

Authors:

Saba Mahjourimajd (saba.mahjourimajd@adelaide.edu.au)

Dr. Julian Taylor (Julian.taylor@adelaide.edu.au)

Prof. Zed Rengel (zed.rengel@uwa.edu.au)

Dr. Hossein Khabaz-Saberi (Hossein.khabaz-saberi@uwa.edu.au)

Dr. Haydn Kuchel (haydn.kuchel@ausgraintech.com.au)

Dr. Mamoru Okamoto (mamoru.okamoto@acpfg.com.au)

Prof. Peter Langridge (peter.langridge@adelaide.edu.au)

Corresponding author: Peter Langridge, e-mail: peter.langridge@adelaide.edu.au

Office phone: +61 8 83137368

Fax No: +61 8 8313 7102

Abbreviations

Grain protein concentration	GPC
Protein yield	PY
Nitrogen	N
Nitrogen use efficiency	NUE
Doubled haploid	DH
Grain yield	GY
Quantitative Trait Loci	QTL
Best Linear Unbiased Prediction	BLUP
N Responsive grain protein concentration	NRGPC
N Responsive protein yield	NRPY
Genotype	G

Single Nucleotide Polymorphism	SNP
centiMorgans	cM
Logarithm of the Odds	LOD
Composite interval mapping	CIM

Introduction

Nitrogen (N) is one of the most important nutrients for ensuring both high grain yield (GY) and grain quality, and increasing yield and protein content are major objectives for wheat breeding programs. The first element in improving these traits is identification of useful genetic diversity but since the environmental conditions will exert a major influence on the genotypic performance, these must be closely defined or controlled. Nitrogen use efficiency (NUE) is a complex trait and is under the control of multiple genes and is highly influenced by the interaction of the genotype with the environment. To improve genetic performance, we need to assess the significance of NUE compared to the various other traits undergoing selection. For NUE improvement, high N fertiliser application and deployment of genotypes that can efficiently use the N supplied are recommended (Hirel et al. 2007). Determining the N response of genotypes (Bogard et al. 2011) is one of best approaches for achieving high GY under N fertilisation. However, there is generally a negative correlation between GY and grain protein content (GPC) and this represents an important obstacle for improvement of protein accumulation. Previous studies demonstrated that grain protein deviation (GPD) can be used as a trait for selection to simultaneously improve both GY and GPC in a breeding program (Monaghan et al. 2001; Oury and Godin 2007; Bogard et al. 2010).

Bogard et al. (2011) showed that increasing uptake of N after anthesis was a major factor for increases in GPC. They also demonstrated that enhanced GPC occurred through improved N remobilisation and reutilisation into the grain. The synchronisation of N demand and supply in plants, and the relationship of N supply with other environmental factors will influence

GPC. Poor synchronisation of these processes may intensify the negative relationship between GY and GPC (Bogard et al. 2010).

Genetic variation and genome regions associated with the protein content of wheat grain have been detected in previous studies (Groos et al. 2003; Charmet et al. 2005; Laperche et al. 2007; Fontaine et al. 2009; Bogard et al. 2011; Cormier et al. 2013). Here we take advantage of the recent improvement in genomic resources for wheat to identify QTL associated with protein-related traits at varying N. In contrast to the previous studies, we also characterise these traits in low-yielding environments where nitrogen is applied at sowing and excess biomass, in response to N supply, can exacerbate stress during grain filling.

Materials and methods

Plant material

The response to N application for the quality traits was investigated at varying rates of N input. A population of 156 doubled haploid (DH) lines generated from a cross between the Australian wheat cultivars RAC875 and Kukri was evaluated in a multi-environment study in the Southern Australia between 2011 and 2012 and 148 DH lines were grown in trials in Western Australia in 2013 The genotypes were grown in a split-plot design with partial replicatation. The DH lines were selected for a narrow flowering time window of less than one week to minimise the influence of maturity on performance (Mahjourimajd et al. 2015a).

Field experiments and traits measurements

The DH population, parental lines and some local check varieties were grown in the field at different rates of N application in 2011 to 2013. The rates of N supply were low (no added N fertilisation), half the standard rate for the site and full fertilisation Table 1). Nitrogen, supplied as urea, was applied to the main plots at the rates shown in Table 1. Soil analyses were performed on subsamples of soil by CSBP Future Farm analytical laboratories (Bibra Lake, Australia). Standard management practices for the region were applied at all field trials.

Grain yield (GY, kg ha^{-1}) was measured for all plots. The N concentration in grain was determined using an isotope ratio mass spectrometer (Sercon, Crewe, Cheshire, UK) in PIN 11 then multiplied by 5.7 to calculate grain protein concentration (GPC, %) (Sosulski and Imafidon 1990). At other sites, protein in the harvested and cleaned grain was measured using near infrared spectroscopy (NIR, ZEUTEC SpectraAlyzer 2.0) at all N treatments (protein calibration r^2 is 0.93 with a RMSEP of 0.31, Kuchel personal communication, 2013). Protein production or protein yield (PY, kg ha^{-1}) was calculated from the GPC and GY values for each site. Nitrogen responsive GPC and N responsive PY were calculated by comparing the protein values at higher level of N application with the lower N level.

Genotyping and genetic map was performed as described by Mahjourimajd et al. (2015a).

Statistical analysis

The statistical analyses were divided in two sections, as outlined previously (Mahjourimajd et al. 2015a). In the first step, a multi-treatment-environment trial (MTET) linear mixed model including genetic and non-genetic sources of variation present across the multiple treatments and environments (Smith et al. 2001; Smith et al. 2005) was used to analyse the traits of interest. In addition, the effect of flowering time genes *ppdB1* and *ppdD1* for each treatment by environment combination were minimised and measured in the analyses (Bonneau et al. 2012).

Further, the best linear unbiased predictors (BLUP) for GPC, PY and their responsiveness in the DH genotypes were extracted for all rates of N treatment for each environments (Mahjourimajd et al. 2015a). Heritability was calculated for each N treatment by environment combination using the formula derived in Cullis et al. (2006) (Supplementary Tables 1 and 2). All statistical modelling was conducted using the flexible linear mixed modelling package ASReml-R (Butler et al. 2009) available in the R statistical computing environment (R Development Core Team 2014).

QTL mapping

A total of 15,911 markers including 235 SSR, 160 DArT, 15,508 SNP, 2 ISBP and 6 gene–based markers were assembled into 26 linkage groups and assigned to the 21 chromosomes of wheat using 218 indicative lines in the RAC875 × Kukri mapping population. The total length of the genetic map was estimated at 2864 cM, containing 2356 unique loci and the average distance of 1.23 cM (min=0.1 and max= 48.1 cM) between markers. From the SNP-improved and enriched linkage map, a 'base map' consisting of 1344 markers per cluster of collocated markers was extracted and used for QTL mapping (Supplementary Fig. 1).

Quantitative trait loci analysis was conducted for the trials using the 1333 unique loci of the integrated SSR-DArTs-SNP linkage map, QTL analyses were conducted on the protein-BLUPs of the DH genotypes for each treatment by environment combination as well as the responsiveness traits-BLUPs derived from each two level N treatment combination within each environment. Composite interval mapping (CIM) using WinQTLCart-version 2.5 (Model 6 standard analysis) (Wang et al. 2007) detected QTL for the traits of interest for the mean values of genotypes and using the genetic map of 1344 molecular markers. The co-factors for controlling background effects were selected using a walk speed of 1 cM, five control markers and the default window size of 10 cM. The determination of LOD value thresholds was done with a 1,000 permutation test (Churchill and Doerge 1994) with the experimental type I error set at P = 0.05 significance level. The 'asw' designation for 'Australian Spring Wheat' trait abbreviations and QTL designations were defined using the nomenclature suggested in the wheat catalogue (McIntosh et al. 2003). The illustrations for the genetic maps and genome regions were prepared with the MapChart v2.2 software (Voorrips 2002) (Supplementary Fig. 1).

Results

The results showed that there was genetic variation for most of trials except for GPC at N0 at PIN 11, for PY at N0 at PIN 11 and at NO at LAM 12. Consequently, these trials with their

related responsiveness traits were excluded from the subsequent analyses. The highest broad sense heritability for GPC was calculated at PIN 12 under N application (0.68), and similarly for PY at PIN 11 at high N (0.75) (Supplementary Tables 1 and 2).

Fig. 1 illustrates the trends for GPC and PY under varying N provision for the parental lines at sites with significant response to N for the traits. Both parents showed a strong response to N at all sites (e.g.GPC (%) at high N in PIN 11 and LAM 12). Kukri was consistently low for PY at all levels of N supply at PIN12 and WH13. Fig. 2 demonstrates that there was significant transgressive segregation for GPC in the mapping population. Most of the lines showed substantially higher, and some, lower GPC and PY than either parent (Fig. 2 and Table 2). The negative correlation between GY and GPC across trials in this study can be seen from the results shown in Table 3.

Protein-QTL at high and low N

A total of twenty five putative QTL, twelve for GPC and thirteen for PY, were identified on seventeen chromosomes; 1A, 1B, 2A, 2D, 3A-1, 3A-2, 3B, 3D-2, 4A, 4B, 5A, 5B, 5D, 6A, 7A-1, 7B and 7D, and accounted for between 6 and 20% of the phenotypic variance (Table 4) at both high and low N. The LOD for the detected QTL in this category ranged from 3.3 to 10.5. The QTL with the largest effect for GPC were from RAC875 (positive effect) and for PY from Kukri and mapped to chromosomes 7A-1 and 2A, contributing to 17% and 20% of the total variance, respectively. The alleles from Kukri contributed more than RAC875 to increased PY. Moreover, most of the detected QTL were site-specific and adaptive, occurring at either Southern Australia or Western Australia. The two low N-specific QTL, on 1B and 6A, carrying effective alleles from Kukri, were identified for PY at different sites of Western Australia and explained 12 and 9% of the variation, respectively. There were significant QTL under the high N treatment (either half or full N, mostly at the highest rate) for GPC on 1B, 3D-2, 5B (2 loci with the contribution of both parents) and 5D, explaining in total 45% of the variation and for PY on 1A, 3A-1, 3B, 3D-2, 7B and 7D with corresponding total R^2 of 56% and the positive allele from Kukri. Importantly genome regions on chromosomes 3D-2, 4B,

5A (two close regions) and 6A detected for GPC and more regions on 1A, 2A, 3B, 3D-2, 4A (a close region), 6A, 7A-1, 7B and 7D mapped for PY, were co-located with GY in this population (Mahjourimajd et al. 2015a).

According to the results in this study, the proportion of negative alleles from Kukri was higher than for RAC875 for PY. In addition, there were two significant intervals on chromosome 3B with contrasting parental alleles for PY and two loci each on chromosomes 2D, 5A and 5B with the predominant allele from RAC875 for GPC. The twelve QTL for GPC on chromosomes, 1B, 2D, 3D-2, 4B, 5A, 5B, 5D, 6A and 7A-1, accounted for between 7% and 17% of the variance across all sites except for PIN 11. Notably, the largest explanation of variation (17%) was expressed by a QTL in the marker interval *Ra_c9427_300 − BobWhite_c34551_714* for GPC at WH 13 at high N on 7A-1 with a high LOD score and the effective allele from RAC875. Overall, the most stable QTL were on chromosomes 2D and 5A for GPC and also on 2A for PY, each detected at three sites.

N responsive QTL for protein-related traits

Composite interval mapping detected ten QTL for response to N for the protein-related traits, four QTL for N responsive GPC (NRGPC) and six for N responsive PY (NRPY) with LOD scores ranging from 3.1 (NRPY) to 8.9 (NRGPC) (Table 5). Quantitative trait loci on chromosomes 1B, 2A, 3A-1, 3B, 6B and 7B were identified for NRPY. It should be noted that there were putative regions for NRPY on 1B, *RAC875_rep_c77710_180 − Ku_c7557_633*, 3B, *wPt.7984 − Tdurum_contig42513_886* and also on 7B, *wPt.9887 − BobWhite_c25215_457* and *CAP12_c1816_325 − Kukri_c109962_396*, along with the QTL, *QNRGPC.asw-4B,* delineated by marker *BS00068539_51 − BobWhite_c4818_173*, co-localised with a QTL for GY (Mahjourimajd et al, 2015a). The negative allele came from Kukri for the 1B locus and also for the 7B locus which accounted for the highest genotypic variance, 19%, for NRGPC and NRPY, respectively. Most of the QTL for both NRGPC and NRPY were identified as site-specific QTL.

Mapping identified putative QTL for NRGPC on chromosomes, 1B, 2D, 4B and 5A, with the dominant allele from Kukri. Moreover, the highest LOD, R^2 and allele effect values for NRGPC were associated with the *wsnp_RFL_Contig2403_1927045 – wsnp_Ex_c38849_46284348* QTL on 1B detected at PIN 12. There were some QTL characterised only for GPC, such as QTL on chromosomes 5B and 5D. Similarly, *QPY.asw-3A-2* was identified only for PY, representing N associated QTL with no location for GY. Moreover, the region on 2D for GPC and on 3A1 for both PY and NRPY, were identified only for the protein-related traits.

Discussion

In this study, NUE field trials of an RAC875 × Kukri wheat mapping population were conducted in six sites and QTL analysis of protein-related traits was carried out. The population was selected among the lines that showed fairly uniform maturity to minimise the effects of phenology on performance. The varying rates of N application and interactions with other environmental factors resulted in variable responses to N treatments for the target traits. Similarly, these results were identified for GY in the previous study (Mahjourimajd et al. 2015a). Increasing the amount of N fertiliser resulted in higher GPC and PY in the population and the parents in sites showing variable responses to N (Fig. 1). At PIN 11 with relatively high GY, there was a poor correlation between GY and GPC% (- 0.24). However, the average values for GPC and PY were higher than other sites suggesting there would be value in deploying this site to select for GY and N response. It was concerning that the correlation for the target traits between the sites of the West Australia and other sites was poor. This emphasised the strong environmental effect for protein assessment under varying N. It may be desirable to separately assess the Western Australian sites for N response and protein improvement in breeding programs. Importantly, the results showed transgressive segregation and good variation for the protein-related traits among the population despite of low

differences between the parents (Table 2 and Fig. 2) implying significant opportunity to select for improved lines.

The QTL analysis identified some candidate genome regions corresponded to the protein-related traits and their response to N. In the study, the highest R^2 value corresponded to a region on 2A for PY. In addition, the regions on 3B were identified as QTL for PY, at high and low N levels in both southern and western Australian sites. A similar region on 3B was also identified for NRPY.

The results for N response and N-associated QTL (QTL×N interactions) are less clear. Most of the QTL for protein-related traits were specific to the high levels of N supply. All of the protein-related QTL at high N and responsive-protein QTL were associated with N fertilisation and QTL×N, demonstrating the expected positive response to N fertilisation for protein improvement. Overall, the analysis indicated that Kukri carried desirable alleles for protein production. This coincides with the results for the GY analysis implying Kukri alleles will be important for the selection of the lines for improved N response.

It should be noted that the one region controlling GPC and NRGPC on 2D was co-located with a QTL for maturity detected in the NUE field trial (Supplementary Tables 3 and 4). The QTL, on 2D for GPC are likely due to the effect of flowering and photoperiod sensitivity genes in this population, particularly the interval *RAC875_c24201_984 – wsnp_CAP12_c1503_764765*, on chromosome 2D. Moreover, the interval, wsnp*_Ex_c2389_4479352 − barc0353b,* on 6A detected for GPC and PY was co-located with the region controlling relative maturity at LAM 12. Similarly the regions on 7A-1 for GPC and 7B for NRPY are close to the regions mapped for heading date at YAN 11. These effects on the detection of protein traits could be minimised by selecting an even more uniform sub-population along with including maturity as a variable in the composite interval mapping for the QTL analysis in line with the previous study by Bogard et al. (2011). However, reducing the population size might limit resolution and mask other QTL effects. Protein-specific QTL were identified on chromosomes 1B, 2D, 5A, 5B, 5D, 3A-1 and 3A-2, but showed no

association with regions for GY in this population (Mahjourimajd et al, 2015a). However, a previous study by Bennett et al. (2012) showed co-localised QTL on 1B, 2A and 2D for GY with the protein-QTL in this study. It is well-known that GY and GPC are negatively correlated and this has hampered simultaneous improvement of both yield and protein-related traits in breeding programs. However, the regions on 1A, 2A, 3B, 3D-2, 4A, 4B, 5A, 6A, 7A-1, 7B and 7D appear to represent loci where the negative link between GY and high protein may have been broken, and the regions may contain gene(s) that increase both GY and GPC.

Bogard et al. (2011) detected a pleiotropic effect of the QTL on chromosomes 2D and 7D for GPC and GY and connected this to the N availability after anthesis. In addition, they identified significant QTL for GPC on chromosomes 2A, 2B, 2D, 3A, 3B, 5A and 7D. However the loci on 2A, 2D and 7D also overlapped with flowering time QTL in a winter wheat population under different N regimes in their study (Bogard et al. 2011).

Most of the results of the QTL analysis are consistent with previous studies. For example, Charmet et al. (2005) identified genome regions on chromosomes 6A, 7A and 7D for GPC which are close to regions detected in this study. In another study in wheat, Groos et al. (2003) detected significant QTL for GPC on chromosomes 1A, 2A, 3A, 3B, 4A, 4D, 5B, 6A, 7A and 7D with individual R^2 ranging from 4.2% to 10.4%. These loci aligned with the regions identified for the protein-related traits in this study, except for 4D. Our results are also in line with those in a study by Laperche et al. (2007) for GPC, PY at high and low N and response to N (higher level of N – lower level). For instance, among the list of QTL they described, regions on 2D, 3D, 4B and 5B for GPC and QTL on 1B, 2A and 3B for PY co-localised with the regions underlying protein-related traits in our study. Habash et al. (2007) in a genetic analysis of N use in bread wheat presented significant QTL using CIM and 20 background QTL for grain %N on five chromosomes, 2AS, 4AS, 5BS, 5DL and 7A-centromere, accounting for 6% to 21% of the variance. The regions we identified on 2A and 5B are in approximately the same regions. They also located a coincident QTL on 4A underlying grain %N and glutamine synthetase (GS) (Habash et al. 2007). These coincident

QTL demonstrated that the accumulation of protein in grain may depend on enzyme activities therefore the selection for increased protein expression or enzyme activity may lead to increased protein content. Habash et al. (2007) identified GS activity QTL in leaves at the GS2 locus on chromosome 2AL and suggested this may be coincident with QTL on 2B and 2D homoeologues for soluble protein content. They mapped another gene controlling enzyme activity, GS1 to chromosome 6BL with a monomorphic homoeologue located to 6A. We detected a significant region on 6A for GPC and PY and also on 6B for underlying NRPY. Fontaine et al. (2009), in a genetic study of N-related physiological traits in a bread wheat population, located some QTL for GPC and for GS and glutamine dehydrogenase (GDH) activity on chromosome 4B, and 2B, respectively. These loci were corresponded to the traits of interest in our study. A recent NUE study in wheat by Cormier et al. (2014) identified similar QTL on 5A and 5B for GPC and GPC-QTL on 3B, 4A and 7B were detected for PY in our study.

Conclusion

The aim of this project was to distinguish regions associated with N response for the protein-related traits from protein accumulation in the grain. The genome regions identified in this study suggest that there is a real possibility for improvement for these traits. Many of the results reported here match those of previous researches although the confidence intervals for some of these QTL did not completely overlap. Importantly the QTL analysis for PY and GPC demonstrated the importance of assessing both parameters under varying N and in different environments. In addition, the novel regions identified on chromosomes 1B, 2D, 5A (the first region on 87.1 cM), 5B, 5D, 3A-1 and 3A-2, for the protein-related traits were not associated with differences in GY and are promising candidates for targeting protein traits in breeding programs.

Acknowledgements

This research was funded through grants from the Australian Research Council, the Grains Research and Development Corporation, the Government of South Australia and the University of Adelaide.

Reference

Bennett D, Izanloo A, Reynolds M, Kuchel H, Langridge P, Schnurbusch T (2012) Genetic dissection of grain yield and physical grain quality in bread wheat (*Triticum aestivum* L.) under water-limited environments. Theor Appl Genet 125:255-271

Bogard M, Allard V, Brancourt-Hulmel M, Heumez E, Machet JM, Jeuffroy MH, Gate P, Martre P, Le Gouis J (2010) Deviation from the grain protein concentration–grain yield negative relationship is highly correlated to post-anthesis N uptake in winter wheat. J Exp Bot 61:4303-4312

Bogard M, Jourdan M, Allard V, Martre P, Perretant MR, Ravel C, Heumez E, Orford S, Snape J, Griffiths S (2011) Anthesis date mainly explained correlations between post-anthesis leaf senescence, grain yield, and grain protein concentration in a winter wheat population segregating for flowering time QTLs. J Exp Bot 62:3621-3636

Bonneau J, Taylor J, Parent B, Reynolds M, Feuillet C, Langridge P, Mather D (2012) Mult-environment analysis and fine mapping of a yield related QTL on chromosome 3B of wheat. Theoretical and Applied Genetics, Accepted:747-761

Butler D, Cullis B, Gilmour A, Gogel B (2009) ASReml-R reference manual release, 3 edn. Queensland Department of Primary Industries. Technical report,

Charmet G, Robert N, Branlard G, Linossier L, Martre P, Triboï E (2005) Genetic analysis of dry matter and nitrogen accumulation and protein composition in wheat kernels. Theor Appl Genet 111:540-550

Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138:963-971

Cormier F, Faure S, Dubreuil P, Heumez E, Beauchêne K, Lafarge S, Praud S, Le Gouis J (2013) A multi-environmental study of recent breeding progress on nitrogen use efficiency in wheat (*Triticum aestivum* L.). Theor Appl Genet 126:3035-3048

Cormier F, Le Gouis J, Dubreuil P, Lafarge S, Praud S (2014) A genome-wide identification of chromosomal regions determining nitrogen use efficiency components in wheat (Triticum aestivum L.). Theor Appl Genet:1-15

Cullis BR, Smith AB, Coombes NE (2006) On the design of early generation variety trials with correlated data. J Agric Biol Environ Stat 11:381-393

Fontaine J-X, Ravel C, Pageau K, Heumez E, Dubois F, Hirel B, Le Gouis J (2009) A quantitative genetic study for elucidating the contribution of glutamine synthetase, glutamate dehydrogenase and other nitrogen-related physiological traits to the agronomic performance of common wheat. Theor Appl Genet 119:645-662

Groos C, Robert N, Bervas E, Charmet G (2003) Genetic analysis of grain protein-content, grain yield and thousand-kernel weight in bread wheat. Theor Appl Genet 106:1032-1040

Habash DZ, Bernard S, Schondelmaier J, Weyen J, Quarrie SA (2007) The genetics of nitrogen use in hexaploid wheat: N utilisation, development and yield. Theor Appl Genet 114:403-419

Hirel B, Le Gouis J, Ney B, Gallais A (2007) The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. J Exp Bot 58:2369-2387

Laperche A, Brancourt-Hulmel M, Heumez E, Gardet O, Hanocq E, Devienne-Barret F, Le Gouis J (2007) Using genotype× nitrogen interaction variables to evaluate the QTL involved in wheat tolerance to nitrogen constraints. Theor Appl Genet 115:399-415

McIntosh R, Yamazaki Y, Devos K, Dubcovsky J, Rogers W, Appels R Catalogue of gene symbols for wheat. In: 10th International Wheat Genetics Symposium. Paestum (Italie), 2003.

Monaghan JM, Snape JW, Chojecki AJS, Kettlewell PS (2001) The use of grain protein deviation for identifying wheat cultivars with high grain protein concentration and yield. Euphytica 122:309-317

Oury F-X, Godin C (2007) Yield and grain protein concentration in bread wheat: how to use the negative relationship between the two characters to identify favourable genotypes? Euphytica 157:45-57

R Development Core Team (2014) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, ISBN: 3-900051-07-0.

Smith A, Cullis B, Thompson R (2001) Analyzing variety by environment data using multiplicative mixed models and adjustments for spatial field trend. Biometrics 57:1138-1147

Smith A, Cullis BR, Thompson R (2005) The analysis of crop cultivar breeding and evaluation trials: an overview of current mixed model approaches. J Agric Sci 143:449-462

Sosulski FW, Imafidon GI (1990) Amino acid composition and nitrogen-to-protein conversion factors for animal and plant foods. J Agric Food Chem 38:1351-1356

Voorrips R (2002) MapChart: software for the graphical presentation of linkage maps and QTLs. J Hered 93:77-78

Wang S, Basten C, Zeng Z (2007) Windows QTL cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC

Table 1 The location, climatic and basic soil characteristics, growing conditions and average grain yield (GY, kg ha^{-1}) of the South Australia (SA) and Western Australia (WA) in 2011-2013

Site	Year	Abbreviation	Lat[a] (°S)	Lon[b] (°E)	Elv[c] (m)	Total rain[d] (mm)	Hot day[e] (d)	Soil texture[f]	pH level ($CaCl_2$)	pH level (H_2O)	NH_4^+ nitrogen (mg kg^{-1})	NO_3^- nitrogen (mg kg^{-1})	N fertiliser rates (kg ha^{-1})	Average grain yield (kg ha^{-1})
PINERY-SA	2011	PIN 11	34.2	138.6	260	165	16	Clay	7.6	8.2	3	36	0− 75− 150	2236
YANCO-SA	2011	YAN 11	34.6	146.4	164	221	22	n.a.	n.a.	n.a.	n.a.	n.a.	0− 75− 150	1805
LAMEROO-SA	2012	LAM 12	35.3	140.5	99	144	15	Loamy	8.2	9	2	8	18− 52− 87	2007
PINERY-SA	2012	PIN 12	34.2	138.6	260	185	23	Clay	7.7	8.5	3	54	0− 75− 150	2112
ESPERANCE DOWN-WA	2013	ED 13	33.6	121.8	158	293	8	Loamy-sand	5.7	6.3	3	25	0− 60	3065
WONGAN HILLS- WA	2013	WH 13	30.8	116.7	305	163	26	Loamy-sand	6.5	6.9	4	22	0− 35	2559

n.a. data not available

[a] Latitude (Lat °S)
[b] Longitude (Lon °E)
[c] Elevation above sea level (Elv, m)
[d] Total rainfall during growth season
[e] Number of growth season hot days with temperature higher than 30 °C
[f] Soil characteristics at top 10 cm depth of soil before fertilisation

Table 2 Phenotypic performance of RAC875/Kukri population for protein-related traits at Australian sites

Site and year	Parents		Doubled haploid population		
	RAC875	Kukri	Mean	Max	Min
	GPC (%)				
PIN 11	12.0	13.0	13.0	33.9	7.5
YAN 11	14.6	15.5	15.0	18.5	10.0
LAM 12	10.6	11.0	11.2	15.3	7.8
PIN 12	11.6	11.3	11.8	15.6	8.6
ED 13	12.7	13.8	12.9	16.1	9.2
WH 13	11.8	11.1	11.8	14.4	8.9
	PY (kg ha^{-1})				
PIN 11	307.2	316.9	292.9	676	39.5
YAN 11	258.6	287.1	270.2	436.5	124.5
LAM 12	210.2	240.8	218.1	423.9	75.3
PIN 12	275.9	229.3	250.5	402.5	134.2
ED 13	401.6	446.7	391.3	625.1	152.2
WH 13	320.3	277.9	291.0	414.4	93.8

Maximum and minimum of population were calculated across all treatments

Table 3 Phenotypic correlation coefficients of grain yield (GY, kg ha^{-1}) and grain protein concentration (GPC, %) for all genotypes, parental lines and doubled haploid lines, in nitrogen use efficiency field trials of South Australia, 2011-2012

Site and year-Trait-N level	PIN11 GPC N75	PIN11 GPC N150	PIN12 GPC N0	PIN12 GPC N75	PIN12 GPC N150	LAM12 GPC N18	LAM12 GPC N52	LAM12 GPC N87	PIN11 GY N0	PIN11 GY N75	PIN11 GY N150	PIN12 GY N0	PIN12 GY N150	LAM12 GY N52
PIN11 GPC N150	0.08													
PIN12 GPC N0	-0.01	0.04												
PIN12 GPC N75	-0.01	0.07	0.56											
PIN12 GPC N150	0.00	0.07	0.39	0.40										
LAM12 GPC N18	0.12	0.05	0.17	0.26	0.25									
LAM12 GPC N52	-0.02	0.00	0.39	0.38	0.25	0.20								
LAM12 GPC N87	-0.03	0.10	0.10	0.05	0.18	0.22	0.17							
PIN11 GY N0	-0.27	-0.06	0.02	-0.07	-0.03	-0.16	-0.05	0.07						
PIN11 GY N75	-0.30	-0.22	0.03	-0.07	-0.03	-0.10	-0.08	0.06	0.69					
PIN11 GY N150	-0.22	-0.12	-0.01	-0.12	-0.08	-0.08	-0.07	0.13	0.73	0.82				
PIN12 GY N0	-0.02	-0.15	-0.46	-0.32	-0.32	-0.09	-0.24	-0.10	0.22	0.25	0.26			
PIN12 GY N75	-0.01	-0.07	-0.34	-0.35	-0.40	-0.19	-0.25	-0.10	0.21	0.32	0.38	0.51		
PIN12 GY N150	0.04	-0.06	-0.20	-0.33	-0.38	-0.21	-0.06	-0.10	0.05	0.09	0.12	0.25		
LAM12 GY N18	-0.04	-0.08	-0.08	-0.01	0.05	-0.28	-0.07	0.02	0.15	0.13	0.14	0.16	0.03	
LAM12 GY N52	0.04	0.00	-0.24	-0.25	-0.14	-0.22	-0.53	-0.06	0.11	0.09	0.13	0.21	0.13	
LAM12 GY N87	0.07	-0.10	0.08	0.05	0.10	-0.16	0.03	-0.54	0.04	0.10	0.06	0.01	0.11	0.17

Table 4 Genome regions underlying the single effect of nitrogen (N) on protein-related traits, adjoining markers, peak position (cM), logarithm of odd (LOD), R^2 (as %) and additive allele in nitrogen use efficiency field trials of Australia

Chr.	QTL	Trait	N effect	Site and year	Adjoining markers	Position (cM)	LOD	R^2 (%)	Allele effect
1A	1	PY	N150	PIN 11	*Excalibur_c44711_453* − ***Excalibur_c11941_675***	22.9	3.6	6	15.51
1B	2	PY	N0	ED 13	***Excalibur_c1263_901*** − *wsnp_Ku_c4911_8795151*	96.5	5.6	12	-3.89
	3	GPC	N150	PIN 12	*wsnp_Ex_c38849_46284348* − ***stm0658acag***	173.1	4.8	11	-0.16
2A	4	PY	N0	PIN 12	*BS00011893_51* − ***Kukri_c46040_620***	25.7	5.7	12	-2.43
		PY	N150	PIN 11	*BS00011893_51* − ***Kukri_c46040_620***	26.7	3.9	7	-17.42
		PY	N75	PIN 11	*BS00011893_51* − ***Kukri_c46040_620***	26.7	10.5	20	-16.42
		PY	N75	YAN 11	*BS00011893_51* − ***Kukri_c46040_620***	26.7	8.8	17	-11.63
		PY	N150	YAN 11	***Kukri_c46040_620*** − *D_GB5Y7FA02HSMR1_278*	28.7	9	17	-11.43
		PY	N0	YAN 11	***Kukri_c46040_620*** − *D_GB5Y7FA02HSMR1_278*	28.7	8.7	17	-13.74
		PY	N75	PIN 12	*D_GB5Y7FA02HSMR1_278* − ***BobWhite_rep_c64012_389***	40.8	8.2	18	-4.1
2D	5	GPC	N150	PIN 12	***RAC875_c24201_984*** − *wsnp_CAP12_c1503_764765*	39.6	4.4	9	0.16
	6	GPC	N0	YAN 11	*D_GCE8AKX02HFCFH_165* − ***Kukri_c26676_225***	80.4	3.5	8	-0.15
		GPC	N60	ED 13	*RAC875_c39665_175* − ***Ex_c2115_3369***	85.2	4.4	10	-0.09
		GPC	N0	ED 13	*RAC875_c12803_1620* − ***Kukri_c9145_1322***	100.2	4.2	10	-0.11
3A1	7	PY	N60	ED 13	*IAAV1523* − ***wsnp_Ex_c9377_15572157***	15.3	4.1	8	6.99
		PY	N35	WH 13	*IAAV1523* − ***wsnp_Ex_c9377_15572157***	15.3	4.9	11	3.35
3A2	8	PY	N150	YAN 11	*BobWhite_c22778_271* − ***RAC875_s114984_117***	34.3	3.8	6	-6.9
		PY	N0	YAN 11	*BobWhite_c22778_271* − ***RAC875_s114984_117***	34.3	3.8	6	-8.44

Chr.	QTL	Trait	N effect	Site and year	Adjoining markers	Position (cM)	LOD	R^2 (%)	Allele effect
3B	9	PY	N0	WH 13	*wPt.7984* − ***Tdurum_contig42513_886***	5.5	4.4	10	4.68
		PY	N35	WH 13	*wPt.7984* − ***Tdurum_contig42513_886***	5.5	3.9	9	2.93
		PY	N150	PIN 11	*cfb6044* − ***tplb0043c20_1046***	15.4	4.2	8	-16.88
	10	PY	N60	ED 13	***wsnp_Ku_c6387_11197393*** − *wsnp_Ex_c8715_14590273*	73	5.6	11	-8.29
3D2	11	PY	N75	PIN 11	*cfd0064* − ***Excalibur_c3510_1888***	18.7	3.7	6	-9.28
	12	GPC	N60	ED 13	***BS00021930_51*** − *RAC875_c35801_905*	24.3	5.1	11	-0.09
4A	13	PY	N0	PIN 12	*Ku_c24957_677* − ***BobWhite_c12302_389***	144	4.5	9	-2.13
		PY	N75	YAN 11	***BS00093255_51*** − *wPt.6404*	152.6	3.6	6	-7.14
4B	14	GPC	N0	WH 13	*Ku_c2639_1715* − ***GENE.1584_692***	72.7	4.5	9	0.17
		GPC	N35	WH 13	*Ku_c2639_1715* − ***GENE.1584_692***	72.7	4.5	9	0.14
		GPC	N60	ED 13	***BS00004727_51*** − *RFL_Contig5846_1610*	78.1	4.9	10	0.09
5A	15	GPC	N0	YAN 11	*BS00000365_51* − ***BobWhite_c14291_385***	87.1	3.3	7	0.15
	16	GPC	N150	PIN 12	*Excalibur_c49550_97* − ***CAP11_c1685_149***	132.2	4.9	10	0.16
		GPC	N150	YAN 11	***BS00028356_51*** − *BS00022646_51*	154.1	4	8	0.1
		GPC	N75	PIN 12	*BS00022646_51* − ***RAC875_c57603_144***	167.9	4	9	0.14
		GPC	N52	LAM 12	*BS00022867_51* − ***BS00081951_51***	178.2	3.4	7	0.12
5B	17	GPC	N75	PIN 12	***BS00034658_51*** − *BS00032003_51*	0	3.5	8	-0.13
	18	GPC	N150	YAN 11	*BobWhite_c16143_217* − ***BobWhite_c47103_205***	86.9	3.5	7	0.09
5D	19	GPC	N75	YAN 11	*Ku_c1454_984* − ***JD_c16284_736***	51.5	3.8	8	-0.11
		GPC	N35	WH 13	***RAC875_rep_c72023_267*** − *D_GDEEGVY01CEIGE_161*	62	3.4	7	-0.12

Chr.	QTL	Trait	N effect	Site and year	Adjoining markers	Position (cM)	LOD	R^2 (%)	Allele effect
6A	20	GPC	N18	LAM 12	*Kukri_c42078_708* − ***Kukri_c11106_292***	24.2	5.6	12	-0.13
		GPC	N52	LAM 12	*Kukri_c42078_708* − ***Kukri_c11106_292***	24.2	5.4	11	-0.18
		GPC	N87	LAM 12	*Kukri_c42078_708* − ***Kukri_c11106_292***	24.2	5.2	11	-0.12
		GPC	N150	YAN 11	***wsnp_Ex_c2389_4479352*** − *barc0353b*	55.5	3.4	11	-0.11
	21	PY	N0	WH 13	*wsnp_Ex_c2389_4479352* − ***barc0353b***	70.2	4.1	9	-4.62
7A1	22	GPC	N0	WH 13	*Kukri_c60729_430* − ***Ra_c9427_300***	60.5	8.5	17	0.24
		GPC	N35	WH 13	***Ra_c9427_300*** − *BobWhite_c34551_714*	61.4	8.4	17	0.2
	23	PY	N0	PIN 12	***Ku_rep_c104159*** − *Ku_rep_c103889*	105.9	3.4	7	1.84
		PY	N150	YAN 11	*Excalibur_c49272_174* − ***wPt.5558***	114.4	4.7	8	7.85
		PY	N75	YAN 11	*Excalibur_c49272_174* − ***wPt.5558***	114.4	6.2	11	9.64
		PY	N0	YAN 11	*Excalibur_c49272_174* − ***wPt.5558***	114.4	4.7	8	9.69
7B	24	PY	N75	PIN 11	*Kukri_c109962_396* − ***wsnp_Ex_c2103_3947695***	17.4	3.3	6	-8.91
7D	25	PY	N75	PIN 12	***Kukri_c100613_331*** − *RAC875_c53629_483*	83.3	3.8	8	-2.67

Table 5 Genome regions underlying the response to nitrogen (N) for protein-related traits, adjoining markers, peak position (cM), logarithm of odd (LOD), R^2 (as %) and additive allele in nitrogen use efficiency field trials of Australia

Chr.	QTL	Trait	N effect	Site and year	Adjoining markers	Position (cM)	LOD	R^2 (%)	Allele effect
1B	1	NRPY	N150-N0	YAN 11	*RAC875_rep_c77710_180* − ***Ku_c7557_633***	106.4	3.5	7	-0.32
	2	NRGPC	N150-N0	PIN 12	*wsnp_RFL_Contig2403_1927045* − ***wsnp_Ex_c38849_46284348***	172.2	4.4	11	-0.09
		NRGPC	N150-N75	PIN 12	*wsnp_RFL_Contig2403_1927045* − ***wsnp_Ex_c38849_46284348***	172.2	8.9	19	-0.11
		NRGPC	N150-N0	YAN 11	*wsnp_Ex_c38849_46284348* − ***stm0658acag***	173.1	4.3	10	-0.07
		NRGPC	N150-N75	YAN 11	***wsnp_Ex_c38849_46284348*** − *stm0658acag*	173.1	4.1	9	-0.05
2A	3	NRPY	N75-N0	PIN 12	*D_GB5Y7FA02HSMR1_278* − ***BobWhite_rep_c64012_389***	43.3	3.7	7	-1.23
		NRPY	N150-N0	YAN 11	***BobWhite_rep_c64012_389*** − *Ra_c44994_415*	44.8	4.4	9	-0.36
		NRPY	N150-N75	YAN 11	***wsnp_CAP8_c1580_908907*** − *Ku_c23118_149*	48.6	3.3	6	-0.99
2D	4	NRGPC	N150-N0	YAN 11	***RAC875_c24201_984*** − *wsnp_CAP12_c1503_764765*	39.6	4.3	9	0.07
3A1	5	NRPY	N60-N0	ED 13	*IAAV1523* − ***wsnp_Ex_c9377_15572157***	15.3	4.8	11	4.66
3B	6	NRPY	N60-N0	ED 13	*wPt.7984* − ***Tdurum_contig42513_886***	5.5	4	9	4.17
4B	7	NRGPC	N75-N0	PIN 12	*BS00068539_51* − ***BobWhite_c4818_173***	83.1	3.6	9	0.06
		NRGPC	N150-N75	PIN 12	*BS00023024_51* − ***IAAV8499***	88.5	6.7	13	-0.09
5A	8	NRGPC	N150-N0	YAN 11	*Excalibur_c49550_97* − ***CAP11_c1685_149***	132.2	4.4	9	0.07
		NRGPC	N75-N0	YAN 11	*CAP11_c1685_149* − ***Excalibur_c84439_196***	134	3.7	8	0.05
6B	9	NRPY	N87-N52	LAM 12	*Tdurum_contig12397_643* − ***Tdurum_contig61383_627***	21	3.8	9	-9.18
7B	10	NRPY	N150-N75	PIN 12	*wPt.9887* − ***BobWhite_c25215_457***	7.1	3.1	8	5.31
		NRPY	N75-N0	PIN 12	*wsnp_Ra_c3450_6434387* − ***CAP12_c1816_325***	10.3	7.6	16	-1.86
		NRPY	N150-N0	YAN 11	*CAP12_c1816_325* − ***Kukri_c109962_396***	12.3	8.8	19	-0.54
		NRPY	N150-N75	YAN 11	*wsnp_Ra_c31052_40235870* − ***BobWhite_c17355_265***	25.6	4.6	9	-1.21

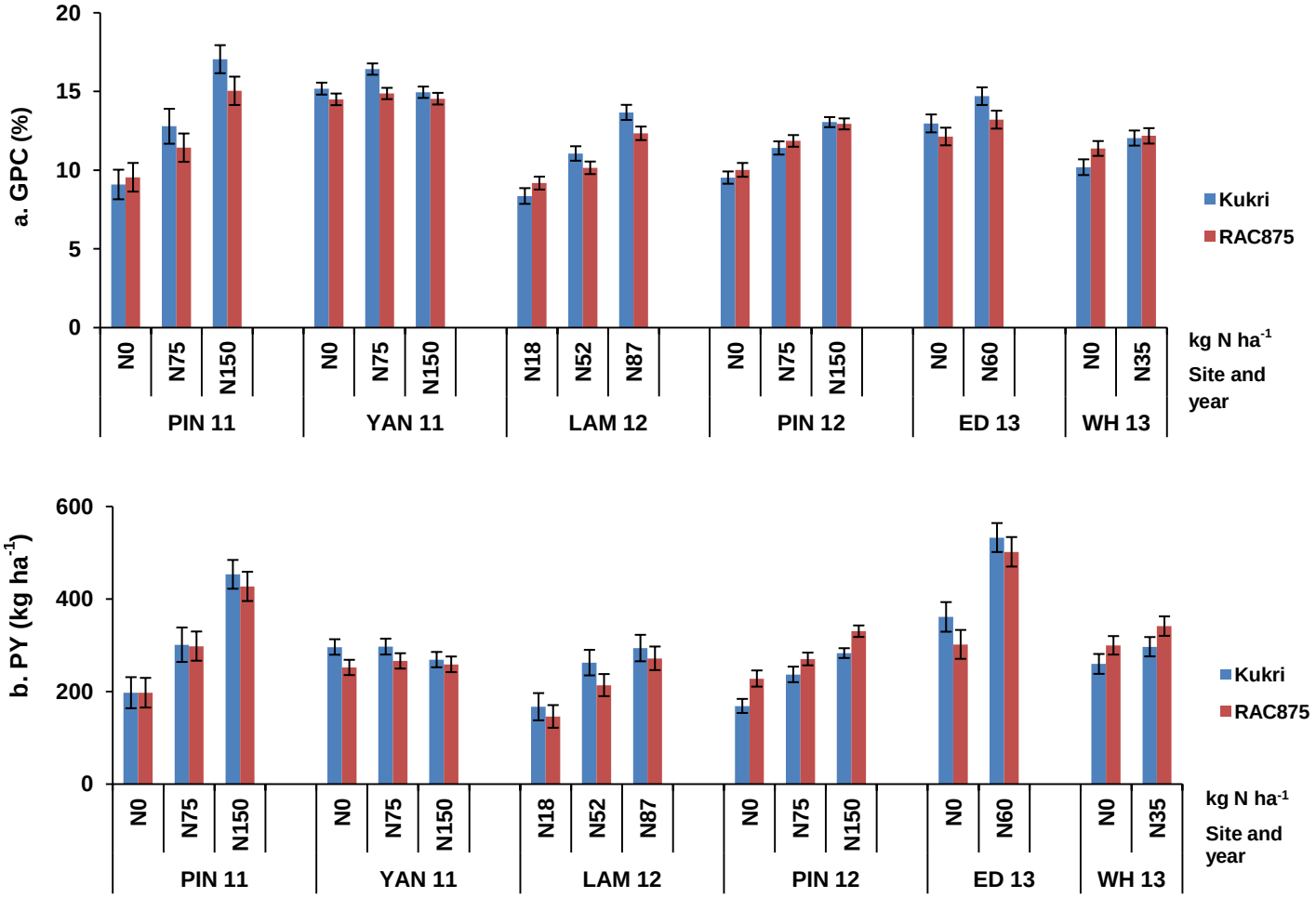


Fig. 1 The performance of parental lines for a. Grain protein concentration (GPC, %) and b. Protein yield (PY, kg ha^{-1}) in Australian sites

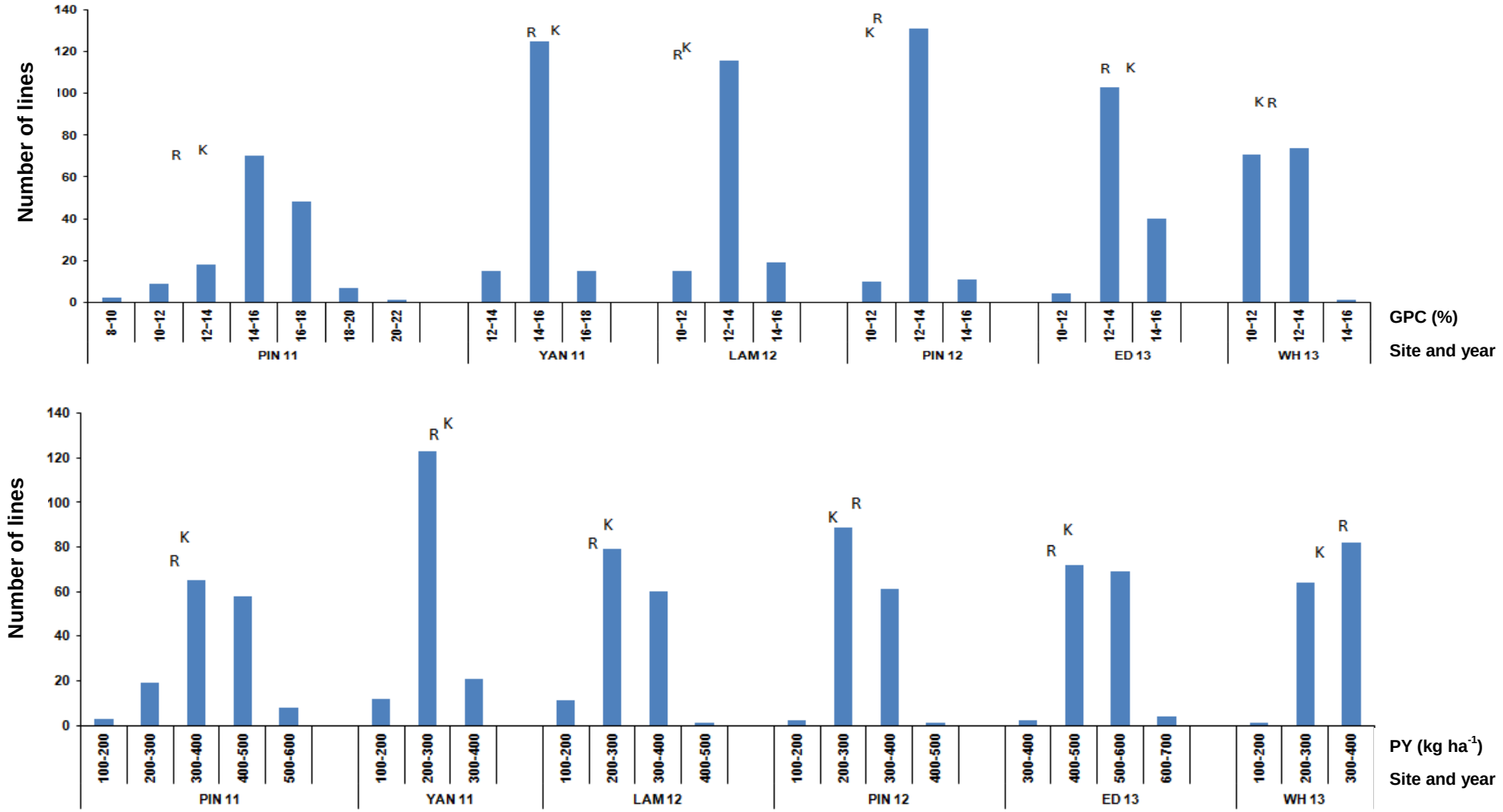


Fig. 2 Distribution of doubled haploid lines for grain protein concentration (GPC, %) and protein yield (PY, kg ha^{-1}) at higher level of nitrogen (N) treatment in Australian sites

Supplementary materials Chapter 5

Table 1 Heritability analysis of the sites for grain protein concentration (GPC, %) at varying nitrogen (N) treatments

Site and year	N treatment	Heritability
PIN11	N0	0
PIN11	N75	0.29
PIN11	N150	0.53
YAN11	N0	0.51
YAN11	N75	0.31
YAN11	N150	0.19
LAM12	N18	0.30
LAM12	N52	0.41
LAM12	N87	0.33
PIN12	N0	0.66
PIN12	N75	0.68
PIN12	N150	0.68
ED13	N0	0.51
ED13	N60	0.60
WH13	N0	0.67
WH13	N35	0.64

Table 2 Heritability analysis of the sites for protein yield (PY, kg ha^{-1}) at varying nitrogen (N) treatments

Site and year	N treatment	Heritability
PIN11	N0	0
PIN11	N75	0.59
PIN11	N150	0.75
YAN11	N0	0.44
YAN11	N75	0.42
YAN11	N150	0.34
LAM12	N18	0
LAM12	N52	0.16
LAM12	N87	0.45
PIN12	N0	0.23
PIN12	N75	0.39
PIN12	N150	0.66
ED13	N0	0.39
ED13	N60	0.54
WH13	N0	0.54
WH13	N35	0.45

Table 3 Genome regions underlying the single effect of nitrogen (N) on heading date (HD), relative anthesis (RA) and relative maturity (RM), adjoining markers, peak position (cM), logarithm of odd (LOD), R^2 (as, %) and additive allele in various Australian sites

Chr.	Trait	N effect	Site and year	Adjoining markers	Position (cM)	LOD	R^2 (%)	Allele effect
2A	RM	N87	LAM 12	*BobWhite_c1049_338* − ***wsnp_Ex_rep_c69799_68760822***	87.2	4.4	10	0.86
2B	RA	N75	PIN 12	***Tdurum_contig54634_956*** − *TA001874.1495*	2.3	5.3	8	0.92
	RM	N52	LAM 12	*wsnp_JD_c23434_20022750* − ***RAC875_c22997_534***	13.7	4.5	9	0.74
2B	HD	N150	YAN 11	*CAP12_c3807_144* − ***Kukri_c26288_419***	21.7	8.7	12	-1.48
	HD	N75	YAN 11	*CAP12_c3807_144* − ***Kukri_c26288_419***	22.7	6.8	9	-1.28
	HD	N0	YAN 11	*CAP12_c3807_144* − ***Kukri_c26288_419***	23.4	7.5	11	-1.48
	RM	N18	LAM 12	*CAP12_c3807_144* − ***Kukri_c26288_419***	23.4	4	8	0.57
2D	RM	N52	LAM 12	*tplb0057n10_689* − ***RAC875_c24201_984***	35.5	10.2	23	-1.27
	HD	N0	YAN 11	*tplb0057n10_689* − ***RAC875_c24201_984***	36.1	16.1	28	2.41
	RA	N0	PIN 12	*tplb0057n10_689* − ***RAC875_c24201_984***	36.1	5.8	13.2	-1.08
	RA	N75	PIN 12	*tplb0057n10_689* − ***RAC875_c24201_984***	36.1	18.2	36	-2.02
	RM	N87	LAM 12	*tplb0057n10_689* − ***RAC875_c24201_984***	36.1	5.6	12	-1.09
	HD	N75	YAN 11	*tplb0057n10_689* − ***RAC875_c24201_984***	37.1	26.5	52	3.06
	HD	N150	YAN 11	*tplb0057n10_689* − ***RAC875_c24201_984***	37.1	28.2	52	3.19
	RA	N150	PIN 12	*tplb0057n10_689* − ***RAC875_c24201_984***	37.1	18.5	37	-2.14
	RM	N18	LAM 12	*tplb0057n10_689* − ***RAC875_c24201_984***	38.1	9.2	21	-0.96
5B	RM	N52	LAM 12	*RAC875_c2260_1274* − ***Ex_c8501_1020***	195.7	4	8	-0.64
6A	RM	N18	LAM 12	***wsnp_Ex_c2389_4479352*** − *barc0353b*	59.5	3.7	10	-0.56
7A1	RA	N0	PIN 12	*Ku_c12886_1250* − ***Excalibur_c15260_94***	47.5	3.7	9.8	0.84
	HD	N150	YAN 11	*Ku_c12886_1250* − ***Excalibur_c15260_94***	52.4	5.3	7	-1.01
	HD	N75	YAN 11	*Ku_c12886_1250* − ***Excalibur_c15260_94***	52.8	7	9	-1.12
	HD	N0	YAN 11	*BS00011072_51* − ***wsnp_Ku_c6065_10682531***	71	3.7	5	-0.89
7B	HD	N75	YAN 11	***IACX198*** − *BS00081132_51*	0	3.4	4	-0.74
	HD	N0	YAN 11	***IACX198*** − *BS00081132_51*	2	6.5	10	-1.25

Table 4 Genome regions underlying the response to nitrogen (N) for on heading date (HD), relative anthesis (RA) and relative maturity (RM), adjoining markers, peak position (cM), logarithm of odd (LOD), R^2 (as, %) and additive allele in various Australian sites

Chr.	Trait	N effect	Site and year	Adjoining markers	Position (cM)	LOD	R^2 (%)	Allele effect
1A	HD	N52-N0	LAM 12	*wsnp_Ku_c34659_43981982* − ***gdm0128***	36.4	4.5	11	-0.81
	HD	N150-N75	YAN 11	*Excalibur_rep_c110054_341* − ***Excalibur_c8599_133***	100.3	6.3	15	-0.82
	RA	N150-N0	PIN 12	*Tdurum_contig4885_1870* − ***BobWhite_c12305_959***	118.7	3.5	9	-0.96
2A	RA	N87-N52	LAM 12	***BobWhite_c1049_338*** − *wsnp_Ex_rep_c69799_68760822*	84.3	3.8	9.23	1.64
	RA	N87-N18	LAM 12	***BobWhite_c1049_338*** − *wsnp_Ex_rep_c69799_68760822*	84.3	3.6	8.6	0.76
2D	RA	N150-N0	PIN 12	*tplb0057n10_689* − ***RAC875_c24201_984***	39.1	4.5	10	-1.14
	RA	N75-N0	PIN 12	*wsnp_CAP12_c1503_764765* − ***Ex_c10377_845***	55	5.8	14	-1.17
4A	RM	N150-N75	PIN 12	*BS00022839_51* − ***Ex_c66324_1151***	65.4	4.2	10	0.75
4B	RM	N75-N0	PIN 12	***Kukri_c26488_139*** − *Excalibur_c64418_447*	19.2	3.6	8	-1.25

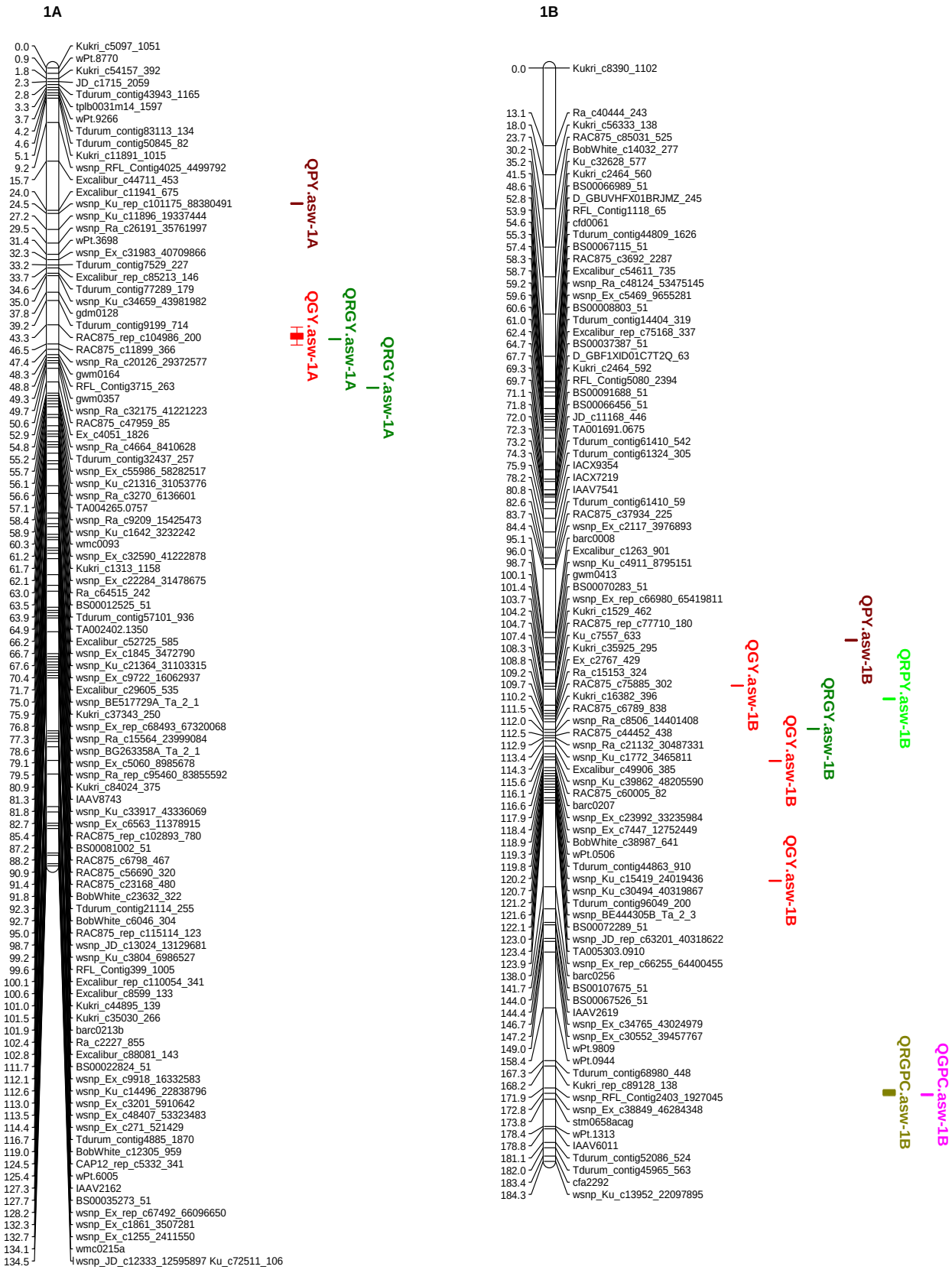
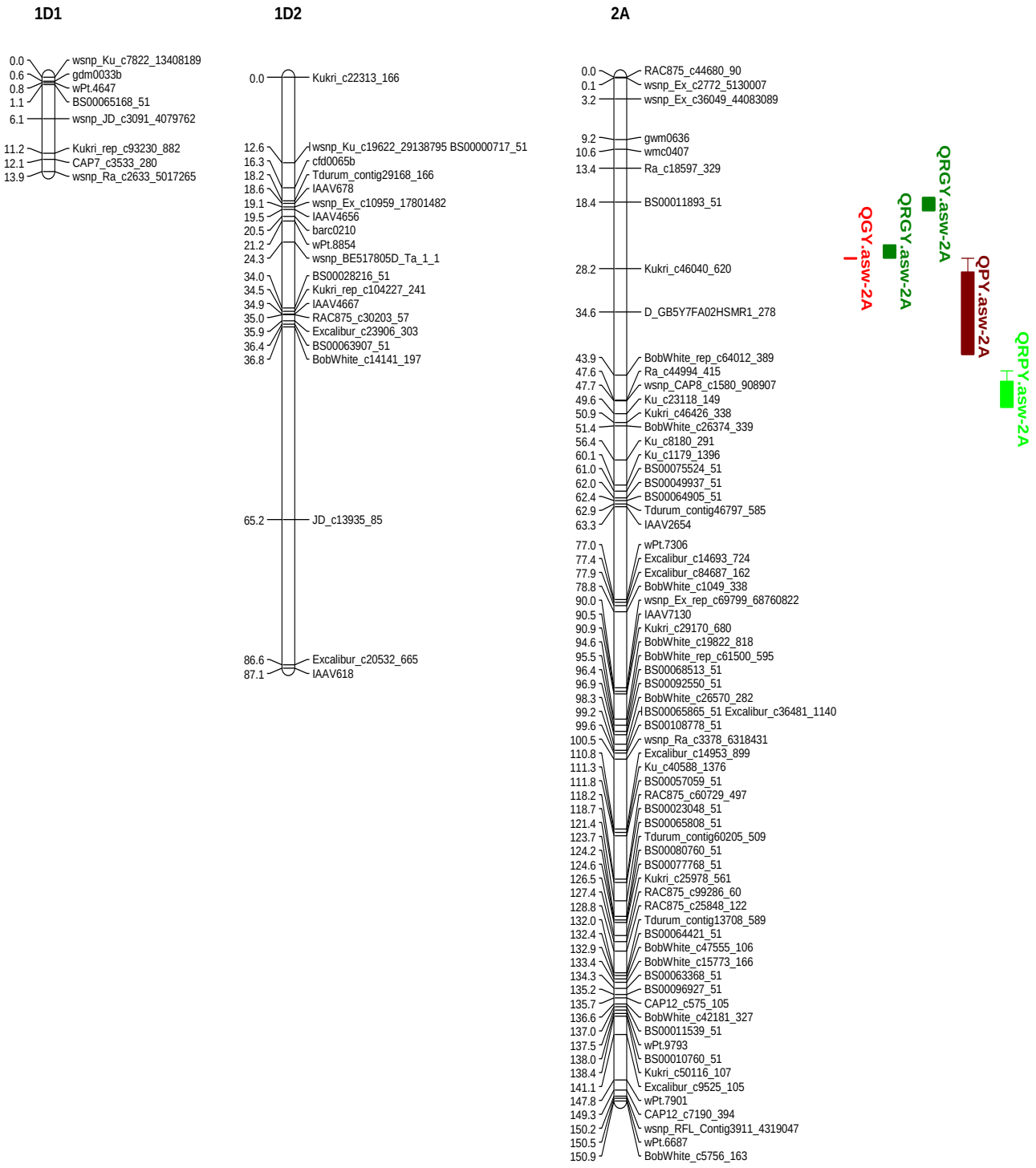
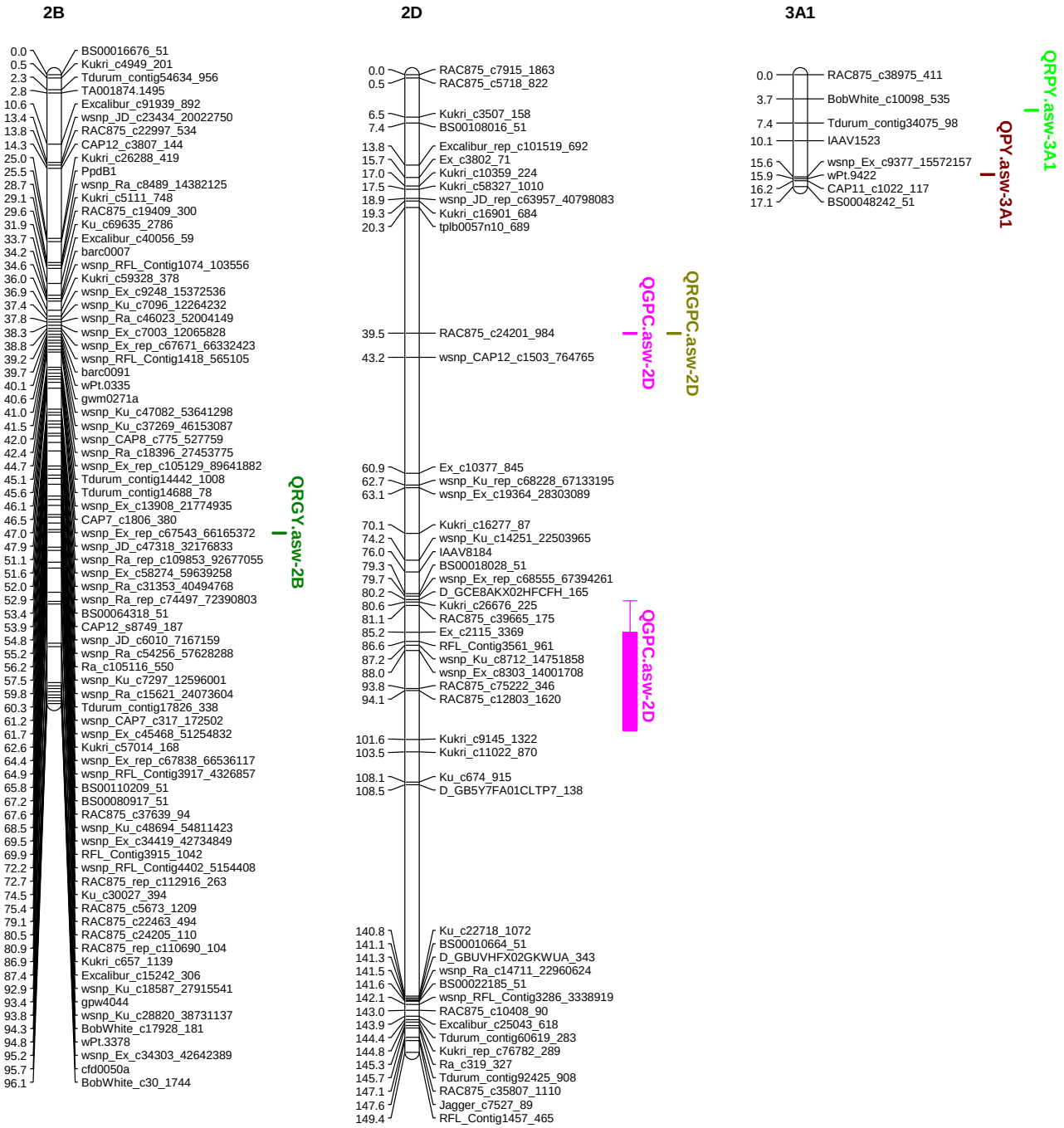
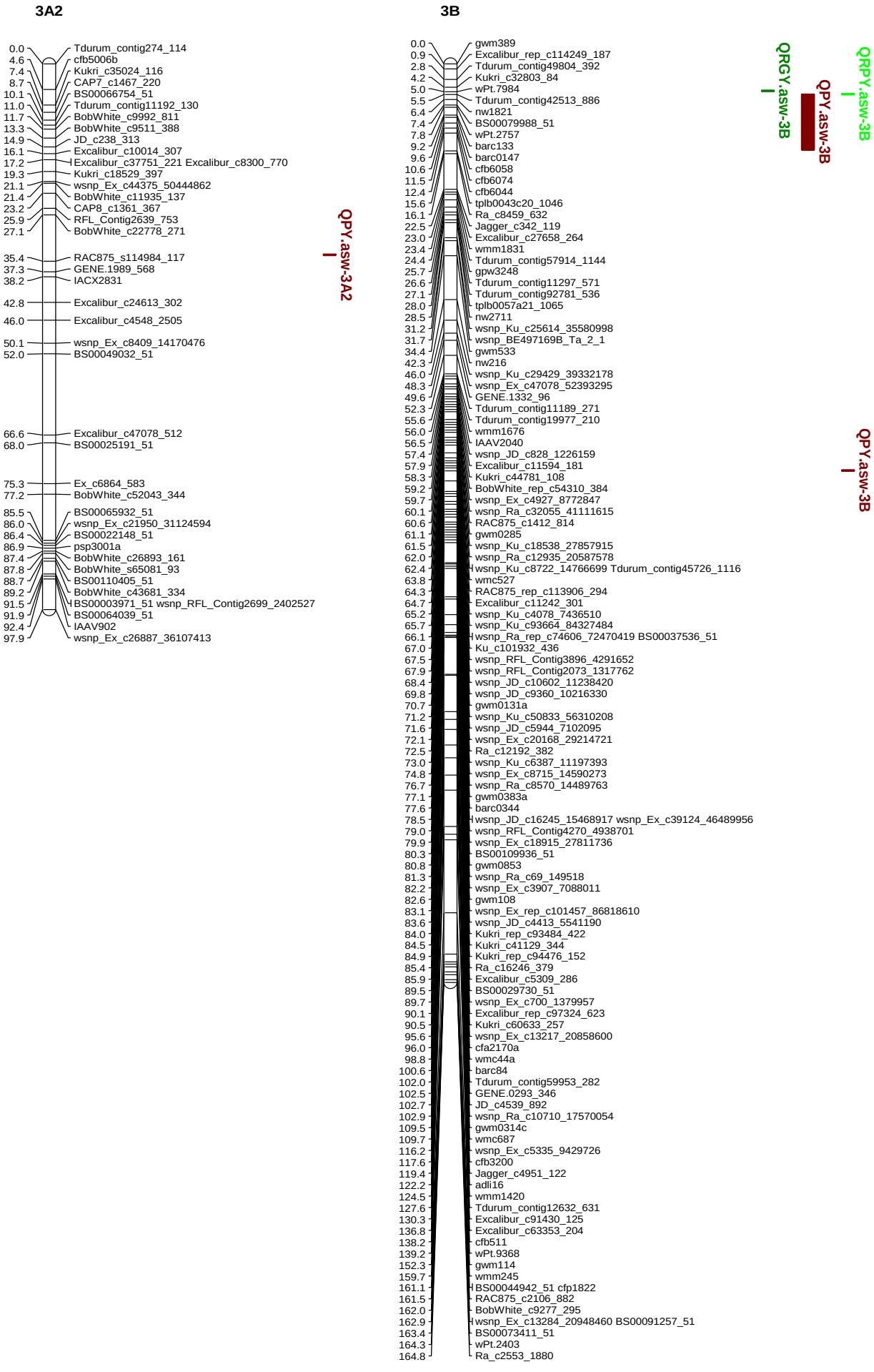
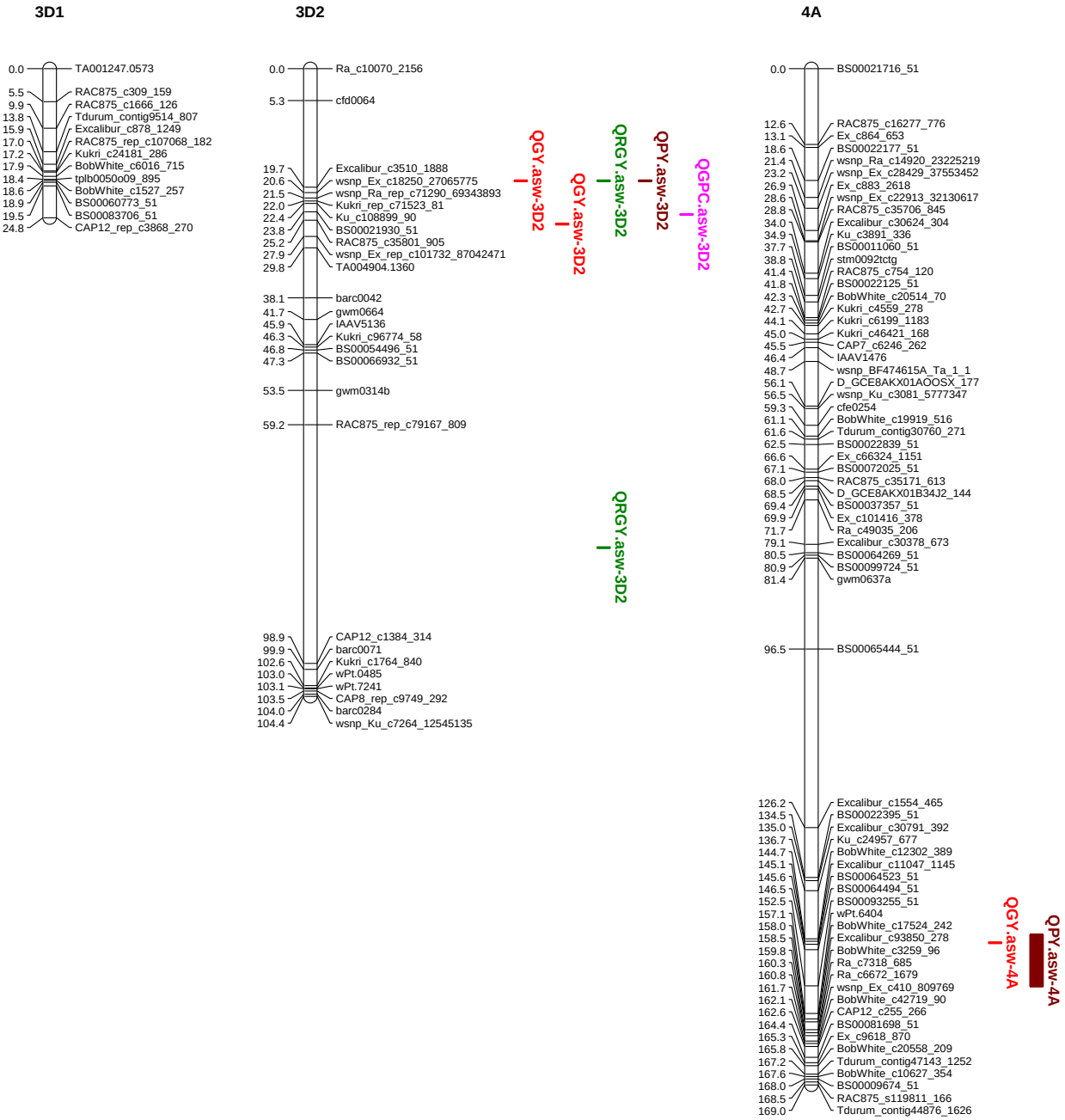


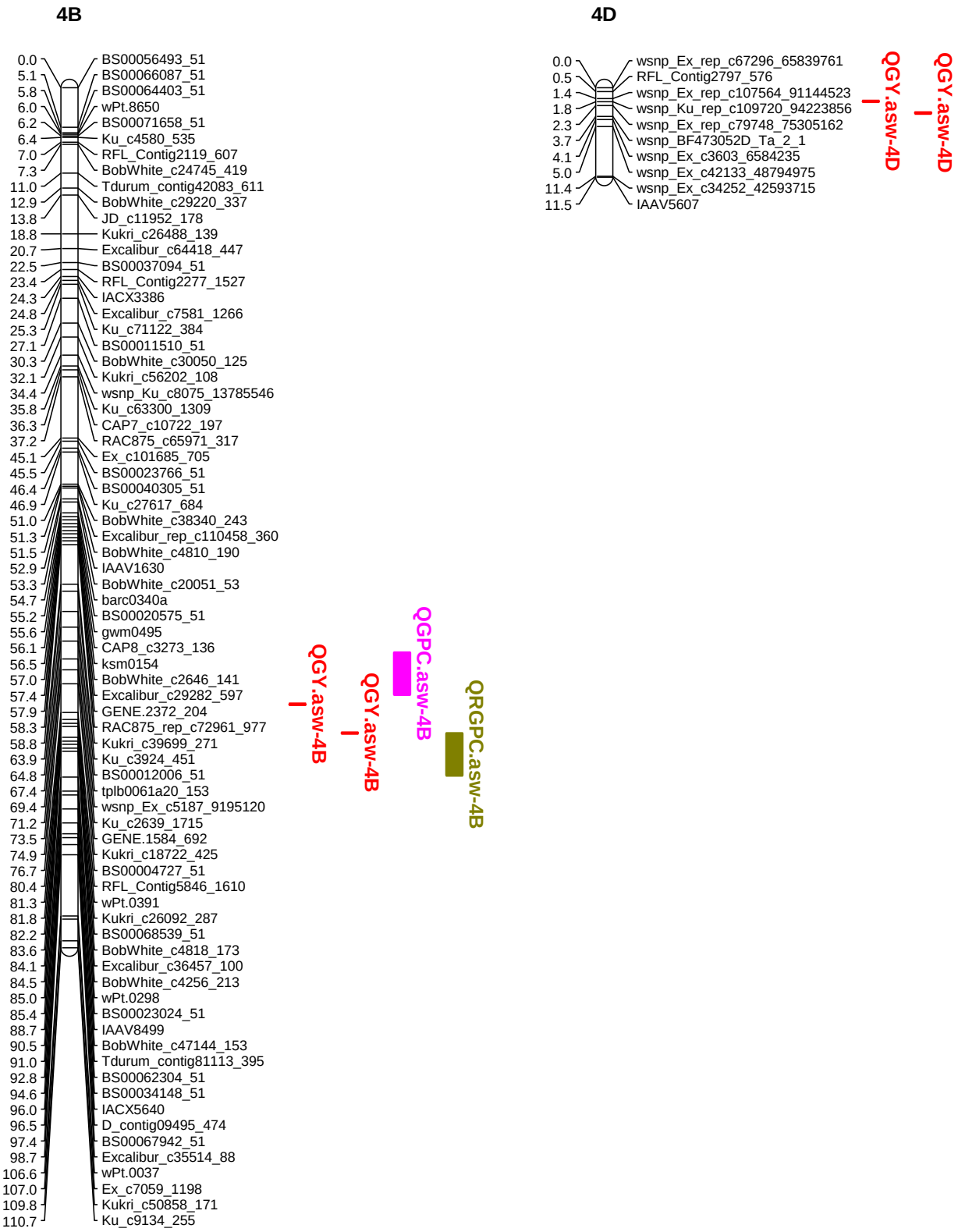
Fig. 1 Significant QTL and markers for grain yield (GY), response to N level for GY (RGY), grain protein concentration (GPC), protein yield (PY) and their response to nitrogen fertiliser.
Distances are in cM

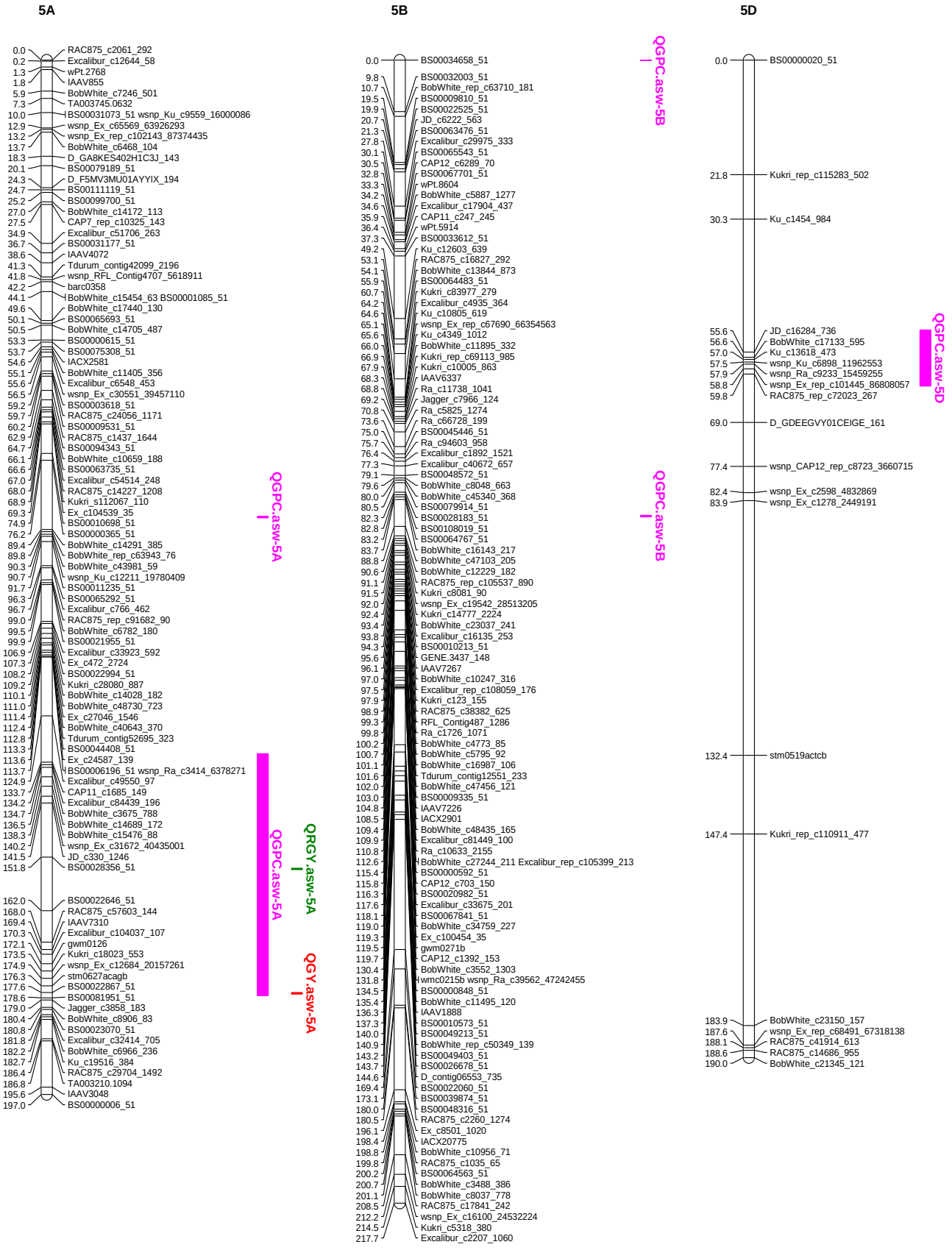

1D1
0.0 wsnp_Ku_c7822_13408189
0.6 gdm0033b
0.8 wPt.4647
1.1 BS00065168_51
6.1 wsnp_JD_c3091_4079762
11.2 Kukri_rep_c93230_882
12.1 CAP7_c3533_280
13.9 wsnp_Ra_c2633_5017265
1D2
0.0 Kukri_c22313_166
12.6 wsnp_Ku_c19622_29138795 BS00000717_51
16.3 cfd0065b
18.2 Tdurum_contig29168_166
18.6 IAAV678
19.1 wsnp_Ex_c10959_17801482
19.5 IAAV4656
20.5 barc0210
21.2 wPt.8854
24.3 wsnp_BE517805D_Ta_1_1
34.0 BS00028216_51
34.5 Kukri_rep_c104227_241
34.9 IAAV4667
35.0 RAC875_c30203_57
35.9 Excalibur_c23906_303
36.4 BS00063907_51
36.8 BobWhite_c14141_197
65.2 JD_c13935_85
86.6 Excalibur_c20532_665
87.1 IAAV618
2A
0.0 RAC875_c44680_90
0.1 wsnp_Ex_c2772_5130007
3.2 wsnp_Ex_c36049_44083089
9.2 gwm0636
10.6 wmc0407
13.4 Ra_c18597_329
18.4 BS00011893_51
28.2 Kukri_c46040_620
34.6 D_GB5Y7FA02HSMR1_278
43.9 BobWhite_rep_c64012_389
47.6 Ra_c44994_415
47.7 wsnp_CAP8_c1580_908907
49.6 Ku_c23118_149
50.9 Kukri_c46426_338
51.4 BobWhite_c26374_339
56.4 Ku_c8180_291
60.1 Ku_c1179_1396
61.0 BS00075524_51
62.0 BS00049937_51
62.4 BS00064905_51
62.9 Tdurum_contig46797_585
63.3 IAAV2654
77.0 wPt.7306
77.4 Excalibur_c14693_724
77.9 Excalibur_c84687_162
78.8 BobWhite_c1049_338
90.0 wsnp_Ex_rep_c69799_68760822
90.5 IAAV7130
90.9 Kukri_c29170_680
94.6 BobWhite_c19822_818
95.5 BobWhite_rep_c61500_595
96.4 BS00068513_51
96.9 BS00092550_51
98.3 BobWhite_c26570_282
99.2 BS00065865_51 Excalibur_c36481_1140
99.6 BS00108778_51
100.5 wsnp_Ra_c3378_6318431
110.8 Excalibur_c14953_899
111.3 Ku_c40588_1376
111.8 BS00057059_51
118.2 RAC875_c60729_497
118.7 BS00023048_51
121.4 BS00065808_51
123.7 Tdurum_contig60205_509
124.2 BS00080760_51
124.6 BS00077768_51
126.5 Kukri_c25978_561
127.4 RAC875_c99286_60
128.8 RAC875_c25848_122
132.0 Tdurum_contig13708_589
132.4 BS00064421_51
132.9 BobWhite_c47555_106
133.4 BobWhite_c15773_166
134.3 BS00063368_51
135.2 BS00096927_51
135.7 CAP12_c575_105
136.6 BobWhite_c42181_327
137.0 BS00011539_51
137.5 wPt.9793
138.0 BS00010760_51
138.4 Kukri_c50116_107
141.1 Excalibur_c9525_105
147.8 wPt.7901
149.3 CAP12_c7190_394
150.2 wsnp_RFL_Contig3911_4319047
150.5 wPt.6687
150.9 BobWhite_c5756_163
QGY.asw-2A
QRGY.asw-2A
QRGY.asw-2A
QPY.asw-2A
QRPY.asw-2A

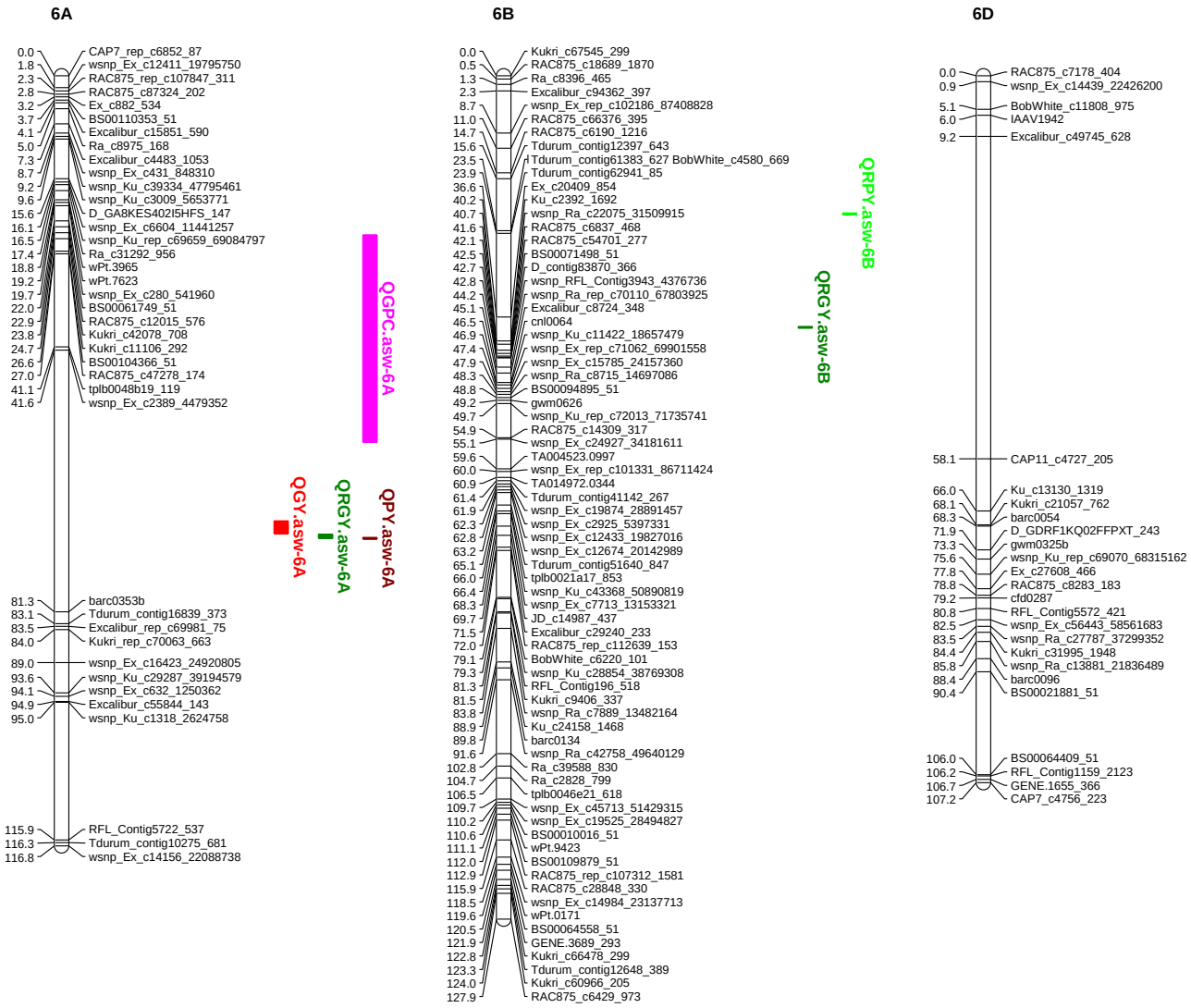

2B
0.0 BS00016676_51
0.5 Kukri_c4949_201
2.3 Tdurum_contig54634_956
2.8 TA001874.1495
10.6 Excalibur_c91939_892
13.4 wsnp_JD_c23434_20022750
13.8 RAC875_c22997_534
14.3 CAP12_c3807_144
25.0 Kukri_c26288_419
25.5 PpdB1
28.7 wsnp_Ra_c8489_14382125
29.1 Kukri_c5111_748
29.6 RAC875_c19409_300
31.9 Ku_c69635_2786
33.7 Excalibur_c40056_59
34.2 barc0007
34.6 wsnp_RFL_Contig1074_103556
36.0 Kukri_c59328_378
36.9 wsnp_Ex_c9248_15372536
37.4 wsnp_Ku_c7096_12264232
37.8 wsnp_Ra_c46023_52004149
38.3 wsnp_Ex_c7003_12065828
38.8 wsnp_Ex_rep_c67671_66332423
39.2 wsnp_RFL_Contig1418_565105
39.7 barc0091
40.1 wPt.0335
40.6 gwm0271a
41.0 wsnp_Ku_c47082_53641298
41.5 wsnp_Ku_c37269_46153087
42.0 wsnp_CAP8_c775_527759
42.4 wsnp_Ra_c18396_27453775
44.7 wsnp_Ex_rep_c105129_89641882
45.1 Tdurum_contig14442_1008
45.6 Tdurum_contig14688_78
46.1 wsnp_Ex_c13908_21774935
46.5 CAP7_c1806_380
47.0 wsnp_Ex_rep_c67543_66165372
47.9 wsnp_JD_c47318_32176833
51.1 wsnp_Ra_rep_c109853_92677055
51.6 wsnp_Ex_c58274_59639258
52.0 wsnp_Ra_c31353_40494768
52.9 wsnp_Ra_rep_c74497_72390803
53.4 BS00064318_51
53.9 CAP12_s8749_187
54.8 wsnp_JD_c6010_7167159
55.2 wsnp_Ra_c54256_57628288
56.2 Ra_c105116_550
57.5 wsnp_Ku_c7297_12596001
59.8 wsnp_Ra_c15621_24073604
60.3 Tdurum_contig17826_338
61.2 wsnp_CAP7_c317_172502
61.7 wsnp_Ex_c45468_51254832
62.6 Kukri_c57014_168
64.4 wsnp_Ex_rep_c67838_66536117
64.9 wsnp_RFL_Contig3917_4326857
65.8 BS00110209_51
67.2 BS00080917_51
67.6 RAC875_c37639_94
68.5 wsnp_Ku_c48694_54811423
69.5 wsnp_Ex_c34419_42734849
69.9 RFL_Contig3915_1042
72.2 wsnp_RFL_Contig4402_5154408
72.7 RAC875_rep_c112916_263
74.5 Ku_c30027_394
75.4 RAC875_c5673_1209
79.1 RAC875_c22463_494
80.5 RAC875_c24205_110
80.9 RAC875_rep_c110690_104
86.9 Kukri_c657_1139
87.4 Excalibur_c15242_306
92.9 wsnp_Ku_c18587_27915541
93.4 gpw4044
93.8 wsnp_Ku_c28820_38731137
94.3 BobWhite_c17928_181
94.8 wPt.3378
95.2 wsnp_Ex_c34303_42642389
95.7 cfd0050a
96.1 BobWhite_c30_1744
QRGY.asw-2B
2D
0.0 RAC875_c7915_1863
0.5 RAC875_c5718_822
6.5 Kukri_c3507_158
7.4 BS00108016_51
13.8 Excalibur_rep_c101519_692
15.7 Ex_c3802_71
17.0 Kukri_c10359_224
17.5 Kukri_c58327_1010
18.9 wsnp_JD_rep_c63957_40798083
19.3 Kukri_c16901_684
20.3 tplb0057n10_689
39.5 RAC875_c24201_984
43.2 wsnp_CAP12_c1503_764765
60.9 Ex_c10377_845
62.7 wsnp_Ku_rep_c68228_67133195
63.1 wsnp_Ex_c19364_28303089
70.1 Kukri_c16277_87
74.2 wsnp_Ku_c14251_22503965
76.0 IAAV8184
79.3 BS00018028_51
79.7 wsnp_Ex_rep_c68555_67394261
80.2 D_GCE8AKX02HFCFH_165
80.6 Kukri_c26676_225
81.1 RAC875_c39665_175
85.2 Ex_c2115_3369
86.6 RFL_Contig3561_961
87.2 wsnp_Ku_c8712_14751858
88.0 wsnp_Ex_c8303_14001708
93.8 RAC875_c75222_346
94.1 RAC875_c12803_1620
101.6 Kukri_c9145_1322
103.5 Kukri_c11022_870
108.1 Ku_c674_915
108.5 D_GB5Y7FA01CLTP7_138
140.8 Ku_c22718_1072
141.1 BS00010664_51
141.3 D_GBUVHFX02GKWUA_343
141.5 wsnp_Ra_c14711_22960624
141.6 BS00022185_51
142.1 wsnp_RFL_Contig3286_3338919
143.0 RAC875_c10408_90
143.9 Excalibur_c25043_618
144.4 Tdurum_contig60619_283
144.8 Kukri_rep_c76782_289
145.3 Ra_c319_327
145.7 Tdurum_contig92425_908
147.1 RAC875_c35807_1110
147.6 Jagger_c7527_89
149.4 RFL_Contig1457_465
QGPC.asw-2D
QRGPC.asw-2D
QGPC.asw-2D
3A1
0.0 RAC875_c38975_411
3.7 BobWhite_c10098_535
7.4 Tdurum_contig34075_98
10.1 IAAV1523
15.6 wsnp_Ex_c9377_15572157
15.9 wPt.9422
16.2 CAP11_c1022_117
17.1 BS00048242_51
QRPY.asw-3A1
QPY.asw-3A1

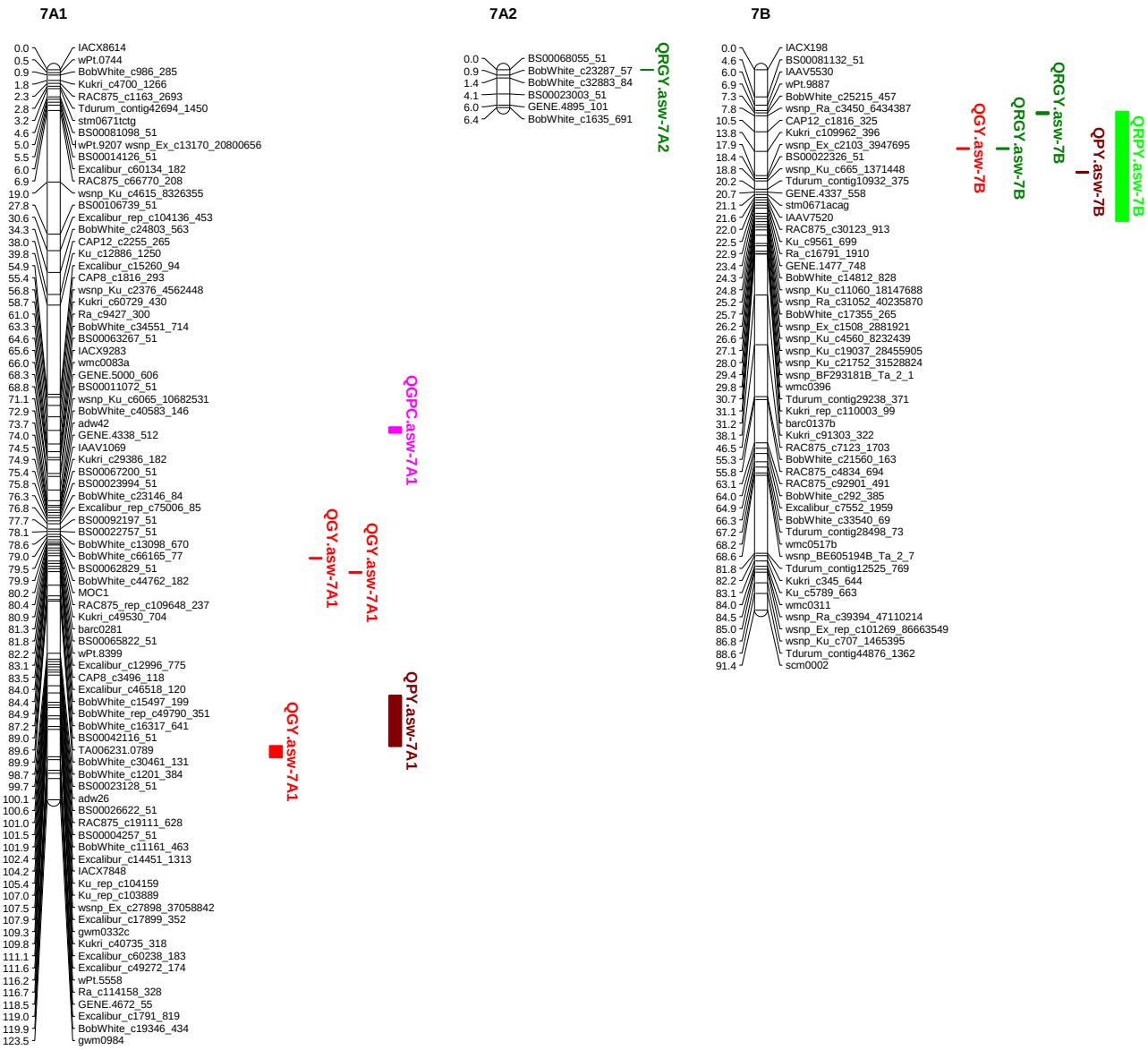

3A2
0.0 Tdurum_contig274_114
4.6 cfb5006b
7.4 Kukri_c35024_116
8.7 CAP7_c1467_220
10.1 BS00066754_51
11.0 Tdurum_contig11192_130
11.7 BobWhite_c9992_811
13.3 BobWhite_c9511_388
14.9 JD_c238_313
16.1 Excalibur_c10014_307
17.2 Excalibur_c37751_221 Excalibur_c8300_770
19.3 Kukri_c18529_397
21.1 wsnp_Ex_c44375_50444862
21.4 BobWhite_c11935_137
23.2 CAP8_c1361_367
25.9 RFL_Contig2639_753
27.1 BobWhite_c22778_271
35.4 RAC875_s114984_117
37.3 GENE.1989_568
38.2 IACX2831
42.8 Excalibur_c24613_302
46.0 Excalibur_c4548_2505
50.1 wsnp_Ex_c8409_14170476
52.0 BS00049032_51
66.6 Excalibur_c47078_512
68.0 BS00025191_51
75.3 Ex_c6864_583
77.2 BobWhite_c52043_344
85.5 BS00065932_51
86.0 wsnp_Ex_c21950_31124594
86.4 BS00022148_51
86.9 psp3001a
87.4 BobWhite_c26893_161
87.8 BobWhite_s65081_93
88.7 BS00110405_51
89.2 BobWhite_c43681_334
91.5 BS00003971_51 wsnp_RFL_Contig2699_2402527
91.9 BS00064039_51
92.4 IAAV902
97.9 wsnp_Ex_c26887_36107413
QPY.asw-3A2
3B
0.0 gwm389
0.9 Excalibur_rep_c114249_187
2.8 Tdurum_contig49804_392
4.2 Kukri_c32803_84
5.0 wPt.7984
5.5 Tdurum_contig42513_886
6.4 nw1821
7.4 BS00079988_51
7.8 wPt.2757
9.2 barc133
9.6 barc0147
10.6 cfb6058
11.5 cfb6074
12.4 cfb6044
15.6 tplb0043c20_1046
16.1 Ra_c8459_632
22.5 Jagger_c342_119
23.0 Excalibur_c27658_264
23.4 wmm1831
24.4 Tdurum_contig57914_1144
25.7 gpw3248
26.6 Tdurum_contig11297_571
27.1 Tdurum_contig92781_536
28.0 tplb0057a21_1065
28.5 nw2711
31.2 wsnp_Ku_c25614_35580998
31.7 wsnp_BE497169B_Ta_2_1
34.4 gwm533
42.3 nw216
46.0 wsnp_Ku_c29429_39332178
48.3 wsnp_Ex_c47078_52393295
49.6 GENE.1332_96
52.3 Tdurum_contig11189_271
55.6 Tdurum_contig19977_210
56.0 wmm1676
56.5 IAAV2040
57.4 wsnp_JD_c828_1226159
57.9 Excalibur_c11594_181
58.3 Kukri_c44781_108
59.2 BobWhite_rep_c54310_384
59.7 wsnp_Ex_c4927_8772847
60.1 wsnp_Ra_c32055_41111615
60.6 RAC875_c1412_814
61.1 gwm0285
61.5 wsnp_Ku_c18538_27857915
62.0 wsnp_Ra_c12935_20587578
62.4 wsnp_Ku_c8722_14766699 Tdurum_contig45726_1116
63.8 wmc527
64.3 RAC875_rep_c113906_294
64.7 Excalibur_c11242_301
65.2 wsnp_Ku_c4078_7436510
65.7 wsnp_Ku_c93664_84327484
66.1 wsnp_Ra_rep_c74606_72470419 BS00037536_51
67.0 Ku_c101932_436
67.5 wsnp_RFL_Contig3896_4291652
67.9 wsnp_RFL_Contig2073_1317762
68.4 wsnp_JD_c10602_11238420
69.8 wsnp_JD_c9360_10216330
70.7 gwm0131a
71.2 wsnp_Ku_c50833_56310208
71.6 wsnp_JD_c5944_7102095
72.1 wsnp_Ex_c20168_29214721
72.5 Ra_c12192_382
73.0 wsnp_Ku_c6387_11197393
74.8 wsnp_Ex_c8715_14590273
76.7 wsnp_Ra_c8570_14489763
77.1 gwm0383a
77.6 barc0344
78.5 wsnp_JD_c16245_15468917 wsnp_Ex_c39124_46489956
79.0 wsnp_RFL_Contig4270_4938701
79.9 wsnp_Ex_c18915_27811736
80.3 BS00109936_51
80.8 gwm0853
81.3 wsnp_Ra_c69_149518
82.2 wsnp_Ex_c3907_7088011
82.6 gwm108
83.1 wsnp_Ex_rep_c101457_86818610
83.6 wsnp_JD_c4413_5541190
84.0 Kukri_rep_c93484_422
84.5 Kukri_c41129_344
84.9 Kukri_rep_c94476_152
85.4 Ra_c16246_379
85.9 Excalibur_c5309_286
89.5 BS00029730_51
89.7 wsnp_Ex_c700_1379957
90.1 Excalibur_rep_c97324_623
90.5 Kukri_c60633_257
95.6 wsnp_Ex_c13217_20858600
96.0 cfa2170a
98.8 wmc44a
100.6 barc84
102.0 Tdurum_contig59953_282
102.5 GENE.0293_346
102.7 JD_c4539_892
102.9 wsnp_Ra_c10710_17570054
109.5 gwm0314c
109.7 wmc687
116.2 wsnp_Ex_c5335_9429726
117.6 cfb3200
119.4 Jagger_c4951_122
122.2 adli16
124.5 wmm1420
127.6 Tdurum_contig12632_631
130.3 Excalibur_c91430_125
136.8 Excalibur_c63353_204
138.2 cfb511
139.2 wPt.9368
152.3 gwm114
159.7 wmm245
161.1 BS00044942_51 cfp1822
161.5 RAC875_c2106_882
162.0 BobWhite_c9277_295
162.9 wsnp_Ex_c13284_20948460 BS00091257_51
163.4 BS00073411_51
164.3 wPt.2403
164.8 Ra_c2553_1880
QRGY.asw-3B
QPY.asw-3B
QRPY.asw-3B
QPY.asw-3B

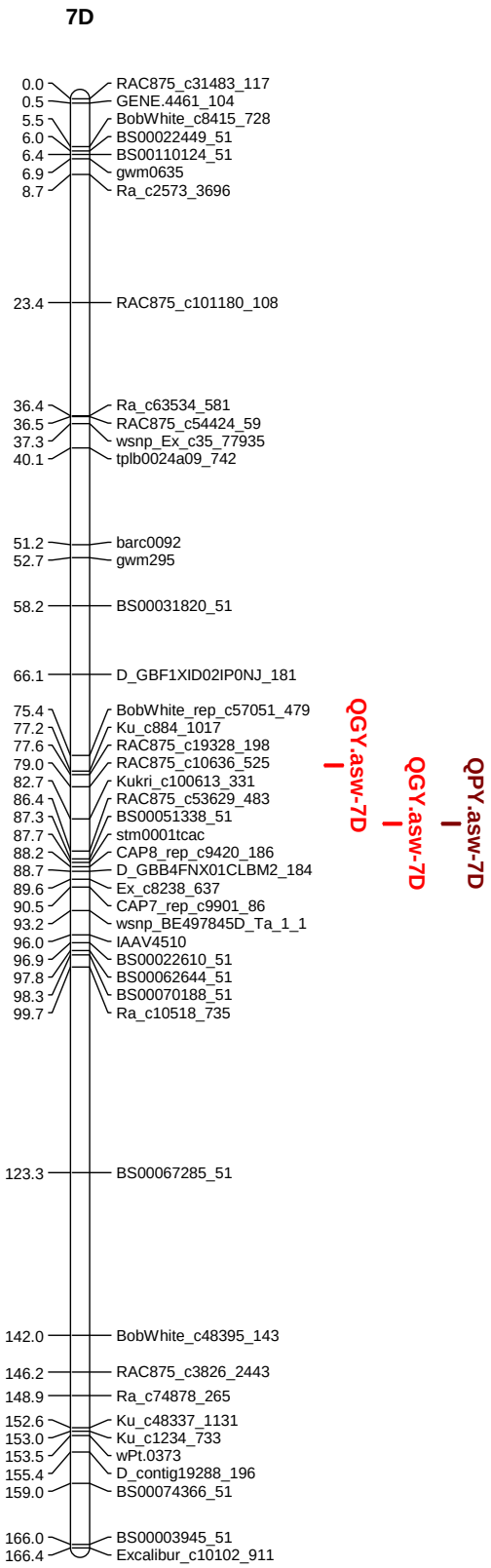

3D1
0.0 TA001247.0573
5.5 RAC875_c309_159
9.9 RAC875_c1666_126
13.8 Tdurum_contig9514_807
15.9 Excalibur_c878_1249
17.0 RAC875_rep_c107068_182
17.2 Kukri_c24181_286
17.9 BobWhite_c6016_715
18.4 tplb0050o09_895
18.6 BobWhite_c1527_257
18.9 BS00060773_51
19.5 BS00083706_51
24.8 CAP12_rep_c3868_270
3D2
0.0 Ra_c10070_2156
5.3 cfd0064
19.7 Excalibur_c3510_1888
20.6 wsnp_Ex_c18250_27065775
21.5 wsnp_Ra_rep_c71290_69343893
22.0 Kukri_rep_c71523_81
22.4 Ku_c108899_90
23.8 BS00021930_51
25.2 RAC875_c35801_905
27.9 wsnp_Ex_rep_c101732_87042471
29.8 TA004904.1360
38.1 barc0042
41.7 gwm0664
45.9 IAAV5136
46.3 Kukri_c96774_58
46.8 BS00054496_51
47.3 BS00066932_51
53.5 gwm0314b
59.2 RAC875_rep_c79167_809
98.9 CAP12_c1384_314
99.9 barc0071
102.6 Kukri_c1764_840
103.0 wPt.0485
103.1 wPt.7241
103.5 CAP8_rep_c9749_292
104.0 barc0284
104.4 wsnp_Ku_c7264_12545135
QGY.asw-3D2
QRGY.asw-3D2
QPY.asw-3D2
QGPC.asw-3D2
QGY.asw-3D2
QRGY.asw-3D2
4A
0.0 BS00021716_51
12.6 RAC875_c16277_776
13.1 Ex_c864_653
18.6 BS00022177_51
21.4 wsnp_Ra_c14920_23225219
23.2 wsnp_Ex_c28429_37553452
26.9 Ex_c883_2618
28.6 wsnp_Ex_c22913_32130617
28.8 RAC875_c35706_845
34.0 Excalibur_c30624_304
34.9 Ku_c3891_336
37.7 BS00011060_51
38.8 stm0092tctg
41.4 RAC875_c754_120
41.8 BS00022125_51
42.3 BobWhite_c20514_70
42.7 Kukri_c4559_278
44.1 Kukri_c6199_1183
45.0 Kukri_c46421_168
45.5 CAP7_c6246_262
46.4 IAAV1476
48.7 wsnp_BF474615A_Ta_1_1
56.1 D_GCE8AKX01AOOSX_177
56.5 wsnp_Ku_c3081_5777347
59.3 cfe0254
61.1 BobWhite_c19919_516
61.6 Tdurum_contig30760_271
62.5 BS00022839_51
66.6 Ex_c66324_1151
67.1 BS00072025_51
68.0 RAC875_c35171_613
68.5 D_GCE8AKX01B34J2_144
69.4 BS00037357_51
69.9 Ex_c101416_378
71.7 Ra_c49035_206
79.1 Excalibur_c30378_673
80.5 BS00064269_51
80.9 BS00099724_51
81.4 gwm0637a
96.5 BS00065444_51
126.2 Excalibur_c1554_465
134.5 BS00022395_51
135.0 Excalibur_c30791_392
136.7 Ku_c24957_677
144.7 BobWhite_c12302_389
145.1 Excalibur_c11047_1145
145.6 BS00064523_51
146.5 BS00064494_51
152.5 BS00093255_51
157.1 wPt.6404
158.0 BobWhite_c17524_242
158.5 Excalibur_c93850_278
159.8 BobWhite_c3259_96
160.3 Ra_c7318_685
160.8 Ra_c6672_1679
161.7 wsnp_Ex_c410_809769
162.1 BobWhite_c42719_90
162.6 CAP12_c255_266
164.4 BS00081698_51
165.3 Ex_c9618_870
165.8 BobWhite_c20558_209
167.2 Tdurum_contig47143_1252
167.6 BobWhite_c10627_354
168.0 BS00009674_51
168.5 RAC875_s119811_166
169.0 Tdurum_contig44876_1626
QGY.asw-4A
QPY.asw-4A


4B
0.0 BS00056493_51
5.1 BS00066087_51
5.8 BS00064403_51
6.0 wPt.8650
6.2 BS00071658_51
6.4 Ku_c4580_535
7.0 RFL_Contig2119_607
7.3 BobWhite_c24745_419
11.0 Tdurum_contig42083_611
12.9 BobWhite_c29220_337
13.8 JD_c11952_178
18.8 Kukri_c26488_139
20.7 Excalibur_c64418_447
22.5 BS00037094_51
23.4 RFL_Contig2277_1527
24.3 IACX3386
24.8 Excalibur_c7581_1266
25.3 Ku_c71122_384
27.1 BS00011510_51
30.3 BobWhite_c30050_125
32.1 Kukri_c56202_108
34.4 wsnp_Ku_c8075_13785546
35.8 Ku_c63300_1309
36.3 CAP7_c10722_197
37.2 RAC875_c65971_317
45.1 Ex_c101685_705
45.5 BS00023766_51
46.4 BS00040305_51
46.9 Ku_c27617_684
51.0 BobWhite_c38340_243
51.3 Excalibur_rep_c110458_360
51.5 BobWhite_c4810_190
52.9 IAAV1630
53.3 BobWhite_c20051_53
54.7 barc0340a
55.2 BS00020575_51
55.6 gwm0495
56.1 CAP8_c3273_136
56.5 ksm0154
57.0 BobWhite_c2646_141
57.4 Excalibur_c29282_597
57.9 GENE.2372_204
58.3 RAC875_rep_c72961_977
58.8 Kukri_c39699_271
63.9 Ku_c3924_451
64.8 BS00012006_51
67.4 tplb0061a20_153
69.4 wsnp_Ex_c5187_9195120
71.2 Ku_c2639_1715
73.5 GENE.1584_692
74.9 Kukri_c18722_425
76.7 BS00004727_51
80.4 RFL_Contig5846_1610
81.3 wPt.0391
81.8 Kukri_c26092_287
82.2 BS00068539_51
83.6 BobWhite_c4818_173
84.1 Excalibur_c36457_100
84.5 BobWhite_c4256_213
85.0 wPt.0298
85.4 BS00023024_51
88.7 IAAV8499
90.5 BobWhite_c47144_153
91.0 Tdurum_contig81113_395
92.8 BS00062304_51
94.6 BS00034148_51
96.0 IACX5640
96.5 D_contig09495_474
97.4 BS00067942_51
98.7 Excalibur_c35514_88
106.6 wPt.0037
107.0 Ex_c7059_1198
109.8 Kukri_c50858_171
110.7 Ku_c9134_255
QGY.asw-4B
QGY.asw-4B
QGPC.asw-4B
QRGPC.asw-4B
4D
0.0 wsnp_Ex_rep_c67296_65839761
0.5 RFL_Contig2797_576
1.4 wsnp_Ex_rep_c107564_91144523
1.8 wsnp_Ku_rep_c109720_94223856
2.3 wsnp_Ex_rep_c79748_75305162
3.7 wsnp_BF473052D_Ta_2_1
4.1 wsnp_Ex_c3603_6584235
5.0 wsnp_Ex_c42133_48794975
11.4 wsnp_Ex_c34252_42593715
11.5 IAAV5607
QGY.asw-4D
QGY.asw-4D


5A
0.0 RAC875_c2061_292
0.2 Excalibur_c12644_58
1.3 wPt.2768
1.8 IAAV855
5.9 BobWhite_c7246_501
7.3 TA003745.0632
10.0 BS00031073_51 wsnp_Ku_c9559_16000086
12.9 wsnp_Ex_c65569_63926293
13.2 wsnp_Ex_rep_c102143_87374435
13.7 BobWhite_c6468_104
18.3 D_GA8KES402H1C3J_143
20.1 BS00079189_51
24.3 D_F5MV3MU01AYYIX_194
24.7 BS00111119_51
25.2 BS00099700_51
27.0 BobWhite_c14172_113
27.5 CAP7_rep_c10325_143
34.9 Excalibur_c51706_263
36.7 BS00031177_51
38.6 IAAV4072
41.3 Tdurum_contig42099_2196
41.8 wsnp_RFL_Contig4707_5618911
42.2 barc0358
44.1 BobWhite_c15454_63 BS00001085_51
49.6 BobWhite_c17440_130
50.1 BS00065693_51
50.5 BobWhite_c14705_487
53.3 BS00000615_51
53.7 BS00075308_51
54.6 IACX2581
55.1 BobWhite_c11405_356
55.6 Excalibur_c6548_453
56.5 wsnp_Ex_c30551_39457110
59.2 BS00003618_51
59.7 RAC875_c24056_1171
60.2 BS00009531_51
62.9 RAC875_c1437_1644
64.7 BS00094343_51
66.1 BobWhite_c10659_188
66.6 BS00063735_51
67.0 Excalibur_c54514_248
68.0 RAC875_c14227_1208
68.9 Kukri_s112067_110
69.3 Ex_c104539_35
74.9 BS00010698_51
76.2 BS00000365_51
89.4 BobWhite_c14291_385
89.8 BobWhite_rep_c63943_76
90.3 BobWhite_c43981_59
90.7 wsnp_Ku_c12211_19780409
91.7 BS00011235_51
96.3 BS00065292_51
96.7 Excalibur_c766_462
99.0 RAC875_rep_c91682_90
99.5 BobWhite_c6782_180
99.9 BS00021955_51
106.9 Excalibur_c33923_592
107.3 Ex_c472_2724
108.2 BS00022994_51
109.2 Kukri_c28080_887
110.1 BobWhite_c14028_182
111.0 BobWhite_c48730_723
111.4 Ex_c27046_1546
112.4 BobWhite_c40643_370
112.8 Tdurum_contig52695_323
113.3 BS00044408_51
113.6 Ex_c24587_139
113.7 BS00006196_51 wsnp_Ra_c3414_6378271
124.9 Excalibur_c49550_97
133.7 CAP11_c1685_149
134.2 Excalibur_c84439_196
134.7 BobWhite_c3675_788
136.5 BobWhite_c14689_172
138.3 BobWhite_c15476_88
140.2 wsnp_Ex_c31672_40435001
141.5 JD_c330_1246
151.8 BS00028356_51
162.0 BS00022646_51
168.0 RAC875_c57603_144
169.4 IAAV7310
170.3 Excalibur_c104037_107
172.1 gwm0126
173.5 Kukri_c18023_553
174.9 wsnp_Ex_c12684_20157261
176.3 stm0627acagb
177.6 BS00022867_51
178.6 BS00081951_51
179.0 Jagger_c3858_183
180.4 BobWhite_c8906_83
180.8 BS00023070_51
181.8 Excalibur_c32414_705
182.2 BobWhite_c6966_236
182.7 Ku_c19516_384
186.4 RAC875_c29704_1492
186.8 TA003210.1094
195.6 IAAV3048
197.0 BS00000006_51
QGPC.asw-5A
QRGY.asw-5A
QGPC.asw-5A
QGY.asw-5A
5B
0.0 BS00034658_51
9.8 BS00032003_51
10.7 BobWhite_rep_c63710_181
19.5 BS00009810_51
19.9 BS00022525_51
20.7 JD_c6222_563
21.3 BS00063476_51
27.8 Excalibur_c29975_333
30.1 BS00065543_51
30.5 CAP12_c6289_70
32.8 BS00067701_51
33.3 wPt.8604
34.2 BobWhite_c5887_1277
34.6 Excalibur_c17904_437
35.9 CAP11_c247_245
36.4 wPt.5914
37.3 BS00033612_51
49.2 Ku_c12603_639
53.1 RAC875_c16827_292
54.1 BobWhite_c13844_873
55.9 BS00064483_51
60.7 Kukri_c83977_279
64.2 Excalibur_c4935_364
64.6 Ku_c10805_619
65.1 wsnp_Ex_rep_c67690_66354563
65.6 Ku_c4349_1012
66.0 BobWhite_c11895_332
66.9 Kukri_rep_c69113_985
67.9 Kukri_c10005_863
68.3 IAAV6337
68.8 Ra_c11738_1041
69.2 Jagger_c7966_124
70.8 Ra_c5825_1274
73.6 Ra_c66728_199
75.0 BS00045446_51
75.7 Ra_c94603_958
76.4 Excalibur_c1892_1521
77.3 Excalibur_c40672_657
79.1 BS00048572_51
79.6 BobWhite_c8048_663
80.0 BobWhite_c45340_368
80.5 BS00079914_51
82.3 BS00028183_51
82.8 BS00108019_51
83.2 BS00064767_51
83.7 BobWhite_c16143_217
88.8 BobWhite_c47103_205
90.6 BobWhite_c12229_182
91.1 RAC875_rep_c105537_890
91.5 Kukri_c8081_90
92.0 wsnp_Ex_c19542_28513205
92.4 Kukri_c14777_2224
93.4 BobWhite_c23037_241
93.8 Excalibur_c16135_253
94.3 BS00010213_51
95.6 GENE.3437_148
96.1 IAAV7267
97.0 BobWhite_c10247_316
97.5 Excalibur_rep_c108059_176
97.9 Kukri_c123_155
98.9 RAC875_c38382_625
99.3 RFL_Contig487_1286
99.8 Ra_c1726_1071
100.2 BobWhite_c4773_85
100.7 BobWhite_c5795_92
101.1 BobWhite_c16987_106
101.6 Tdurum_contig12551_233
102.0 BobWhite_c47456_121
103.0 BS00009335_51
104.8 IAAV7226
108.5 IACX2901
109.4 BobWhite_c48435_165
109.9 Excalibur_c81449_100
110.8 Ra_c10633_2155
112.6 BobWhite_c27244_211 Excalibur_rep_c105399_213
115.4 BS00000592_51
115.8 CAP12_c703_150
116.3 BS00020982_51
117.6 Excalibur_c33675_201
118.1 BS00067841_51
119.0 BobWhite_c34759_227
119.3 Ex_c100454_35
119.5 gwm0271b
119.7 CAP12_c1392_153
130.4 BobWhite_c3552_1303
131.8 wmc0215b wsnp_Ra_c39562_47242455
134.5 BS00000848_51
135.4 BobWhite_c11495_120
136.3 IAAV1888
137.3 BS00010573_51
140.0 BS00049213_51
140.9 BobWhite_rep_c50349_139
143.2 BS00049403_51
143.7 BS00026678_51
144.6 D_contig06553_735
169.4 BS00022060_51
173.1 BS00039874_51
180.0 BS00048316_51
180.5 RAC875_c2260_1274
196.1 Ex_c8501_1020
198.4 IACX20775
198.8 BobWhite_c10956_71
199.8 RAC875_c1035_65
200.2 BS00064563_51
200.7 BobWhite_c3488_386
201.1 BobWhite_c8037_778
208.5 RAC875_c17841_242
212.2 wsnp_Ex_c16100_24532224
214.5 Kukri_c5318_380
217.7 Excalibur_c2207_1060
QGPC.asw-5B
QGPC.asw-5B
5D
0.0 BS00000020_51
21.8 Kukri_rep_c115283_502
30.3 Ku_c1454_984
55.6 JD_c16284_736
56.6 BobWhite_c17133_595
57.0 Ku_c13618_473
57.5 wsnp_Ku_c6898_11962553
57.9 wsnp_Ra_c9233_15459255
58.8 wsnp_Ex_rep_c101445_86808057
59.8 RAC875_rep_c72023_267
69.0 D_GDEEGVY01CEIGE_161
77.4 wsnp_CAP12_rep_c8723_3660715
82.4 wsnp_Ex_c2598_4832869
83.9 wsnp_Ex_c1278_2449191
132.4 stm0519actcb
147.4 Kukri_rep_c110911_477
183.9 BobWhite_c23150_157
187.6 wsnp_Ex_rep_c68491_67318138
188.1 RAC875_c41914_613
188.6 RAC875_c14686_955
190.0 BobWhite_c21345_121
QGPC.asw-5D


6A
6B
6D
0.0 CAP7_rep_c6852_87
1.8 wsnp_Ex_c12411_19795750
2.3 RAC875_rep_c107847_311
2.8 RAC875_c87324_202
3.2 Ex_c882_534
3.7 BS00110353_51
4.1 Excalibur_c15851_590
5.0 Ra_c8975_168
7.3 Excalibur_c4483_1053
8.7 wsnp_Ex_c431_848310
9.2 wsnp_Ku_c39334_47795461
9.6 wsnp_Ku_c3009_5653771
15.6 D_GA8KES402I5HFS_147
16.1 wsnp_Ex_c6604_11441257
16.5 wsnp_Ku_rep_c69659_69084797
17.4 Ra_c31292_956
18.8 wPt.3965
19.2 wPt.7623
19.7 wsnp_Ex_c280_541960
22.0 BS00061749_51
22.9 RAC875_c12015_576
23.8 Kukri_c42078_708
24.7 Kukri_c11106_292
26.6 BS00104366_51
27.0 RAC875_c47278_174
41.1 tplb0048b19_119
41.6 wsnp_Ex_c2389_4479352
81.3 barc0353b
83.1 Tdurum_contig16839_373
83.5 Excalibur_rep_c69981_75
84.0 Kukri_rep_c70063_663
89.0 wsnp_Ex_c16423_24920805
93.6 wsnp_Ku_c29287_39194579
94.1 wsnp_Ex_c632_1250362
94.9 Excalibur_c55844_143
95.0 wsnp_Ku_c1318_2624758
115.9 RFL_Contig5722_537
116.3 Tdurum_contig10275_681
116.8 wsnp_Ex_c14156_22088738
QGPC.asw-6A
QGY.asw-6A
QRGY.asw-6A
QPY.asw-6A
0.0 Kukri_c67545_299
0.5 RAC875_c18689_1870
1.3 Ra_c8396_465
2.3 Excalibur_c94362_397
8.7 wsnp_Ex_rep_c102186_87408828
11.0 RAC875_c66376_395
14.7 RAC875_c6190_1216
15.6 Tdurum_contig12397_643
23.5 Tdurum_contig61383_627 BobWhite_c4580_669
23.9 Tdurum_contig62941_85
36.6 Ex_c20409_854
40.2 Ku_c2392_1692
40.7 wsnp_Ra_c22075_31509915
41.6 RAC875_c6837_468
42.1 RAC875_c54701_277
42.5 BS00071498_51
42.7 D_contig83870_366
42.8 wsnp_RFL_Contig3943_4376736
44.2 wsnp_Ra_rep_c70110_67803925
45.1 Excalibur_c8724_348
46.5 cnl0064
46.9 wsnp_Ku_c11422_18657479
47.4 wsnp_Ex_rep_c71062_69901558
47.9 wsnp_Ex_c15785_24157360
48.3 wsnp_Ra_c8715_14697086
48.8 BS00094895_51
49.2 gwm0626
49.7 wsnp_Ku_rep_c72013_71735741
54.9 RAC875_c14309_317
55.1 wsnp_Ex_c24927_34181611
59.6 TA004523.0997
60.0 wsnp_Ex_rep_c101331_86711424
60.9 TA014972.0344
61.4 Tdurum_contig41142_267
61.9 wsnp_Ex_c19874_28891457
62.3 wsnp_Ex_c2925_5397331
62.8 wsnp_Ex_c12433_19827016
63.2 wsnp_Ex_c12674_20142989
65.1 Tdurum_contig51640_847
66.0 tplb0021a17_853
66.4 wsnp_Ku_c43368_50890819
68.3 wsnp_Ex_c7713_13153321
69.7 JD_c14987_437
71.5 Excalibur_c29240_233
72.0 RAC875_rep_c112639_153
79.1 BobWhite_c6220_101
79.3 wsnp_Ku_c28854_38769308
81.3 RFL_Contig196_518
81.5 Kukri_c9406_337
83.8 wsnp_Ra_c7889_13482164
88.9 Ku_c24158_1468
89.8 barc0134
91.6 wsnp_Ra_c42758_49640129
102.8 Ra_c39588_830
104.7 Ra_c2828_799
106.5 tplb0046e21_618
109.7 wsnp_Ex_c45713_51429315
110.2 wsnp_Ex_c19525_28494827
110.6 BS00010016_51
111.1 wPt.9423
112.0 BS00109879_51
112.9 RAC875_rep_c107312_1581
115.9 RAC875_c28848_330
118.5 wsnp_Ex_c14984_23137713
119.6 wPt.0171
120.5 BS00064558_51
121.9 GENE.3689_293
122.8 Kukri_c66478_299
123.3 Tdurum_contig12648_389
124.0 Kukri_c60966_205
127.9 RAC875_c6429_973
QRPY.asw-6B
QRGY.asw-6B
0.0 RAC875_c7178_404
0.9 wsnp_Ex_c14439_22426200
5.1 BobWhite_c11808_975
6.0 IAAV1942
9.2 Excalibur_c49745_628
58.1 CAP11_c4727_205
66.0 Ku_c13130_1319
68.1 Kukri_c21057_762
68.3 barc0054
71.9 D_GDRF1KQ02FFPXT_243
73.3 gwm0325b
75.6 wsnp_Ku_rep_c69070_68315162
77.8 Ex_c27608_466
78.8 RAC875_c8283_183
79.2 cfd0287
80.8 RFL_Contig5572_421
82.5 wsnp_Ex_c56443_58561683
83.5 wsnp_Ra_c27787_37299352
84.4 Kukri_c31995_1948
85.8 wsnp_Ra_c13881_21836489
88.4 barc0096
90.4 BS00021881_51
106.0 BS00064409_51
106.2 RFL_Contig1159_2123
106.7 GENE.1655_366
107.2 CAP7_c4756_223


7A1
0.0 IACX8614
0.5 wPt.0744
0.9 BobWhite_c986_285
1.8 Kukri_c4700_1266
2.3 RAC875_c1163_2693
2.8 Tdurum_contig42694_1450
3.2 stm0671tctg
4.6 BS00081098_51
5.0 wPt.9207 wsnp_Ex_c13170_20800656
5.5 BS00014126_51
6.0 Excalibur_c60134_182
6.9 RAC875_c66770_208
19.0 wsnp_Ku_c4615_8326355
27.8 BS00106739_51
30.6 Excalibur_rep_c104136_453
34.3 BobWhite_c24803_563
38.0 CAP12_c2255_265
39.8 Ku_c12886_1250
54.9 Excalibur_c15260_94
55.4 CAP8_c1816_293
56.8 wsnp_Ku_c2376_4562448
58.7 Kukri_c60729_430
61.0 Ra_c9427_300
63.3 BobWhite_c34551_714
64.6 BS00063267_51
65.6 IACX9283
66.0 wmc0083a
68.3 GENE.5000_606
68.8 BS00011072_51
71.1 wsnp_Ku_c6065_10682531
72.9 BobWhite_c40583_146
73.7 adw42
74.0 GENE.4338_512
74.5 IAAV1069
74.9 Kukri_c29386_182
75.4 BS00067200_51
75.8 BS00023994_51
76.3 BobWhite_c23146_84
76.8 Excalibur_rep_c75006_85
77.7 BS00092197_51
78.1 BS00022757_51
78.6 BobWhite_c13098_670
79.0 BobWhite_c66165_77
79.5 BS00062829_51
79.9 BobWhite_c44762_182
80.2 MOC1
80.4 RAC875_rep_c109648_237
80.9 Kukri_c49530_704
81.3 barc0281
81.8 BS00065822_51
82.2 wPt.8399
83.1 Excalibur_c12996_775
83.5 CAP8_c3496_118
84.0 Excalibur_c46518_120
84.4 BobWhite_c15497_199
84.9 BobWhite_rep_c49790_351
87.2 BobWhite_c16317_641
89.0 BS00042116_51
89.6 TA006231.0789
89.9 BobWhite_c30461_131
98.7 BobWhite_c1201_384
99.7 BS00023128_51
100.1 adw26
100.6 BS00026622_51
101.0 RAC875_c19111_628
101.5 BS00004257_51
101.9 BobWhite_c11161_463
102.4 Excalibur_c14451_1313
104.2 IACX7848
105.4 Ku_rep_c104159
107.0 Ku_rep_c103889
107.5 wsnp_Ex_c27898_37058842
107.9 Excalibur_c17899_352
109.3 gwm0332c
109.8 Kukri_c40735_318
111.1 Excalibur_c60238_183
111.6 Excalibur_c49272_174
116.2 wPt.5558
116.7 Ra_c114158_328
118.5 GENE.4672_55
119.0 Excalibur_c1791_819
119.9 BobWhite_c19346_434
123.5 gwm0984
QGPC.asw-7A1
QGY.asw-7A1
QGY.asw-7A1
QPY.asw-7A1
QGY.asw-7A1
7A2
0.0 BS00068055_51
0.9 BobWhite_c23287_57
1.4 BobWhite_c32883_84
4.1 BS00023003_51
6.0 GENE.4895_101
6.4 BobWhite_c1635_691
QRGY.asw-7A2
7B
0.0 IACX198
4.6 BS00081132_51
6.0 IAAV5530
6.9 wPt.9887
7.3 BobWhite_c25215_457
7.8 wsnp_Ra_c3450_6434387
10.5 CAP12_c1816_325
13.8 Kukri_c109962_396
17.9 wsnp_Ex_c2103_3947695
18.4 BS00022326_51
18.8 wsnp_Ku_c665_1371448
20.2 Tdurum_contig10932_375
20.7 GENE.4337_558
21.1 stm0671acag
21.6 IAAV7520
22.0 RAC875_c30123_913
22.5 Ku_c9561_699
22.9 Ra_c16791_1910
23.4 GENE.1477_748
24.3 BobWhite_c14812_828
24.8 wsnp_Ku_c11060_18147688
25.2 wsnp_Ra_c31052_40235870
25.7 BobWhite_c17355_265
26.2 wsnp_Ex_c1508_2881921
26.6 wsnp_Ku_c4560_8232439
27.1 wsnp_Ku_c19037_28455905
28.0 wsnp_Ku_c21752_31528824
29.4 wsnp_BF293181B_Ta_2_1
29.8 wmc0396
30.7 Tdurum_contig29238_371
31.1 Kukri_rep_c110003_99
31.2 barc0137b
38.1 Kukri_c91303_322
46.5 RAC875_c7123_1703
55.3 BobWhite_c21560_163
55.8 RAC875_c4834_694
63.1 RAC875_c92901_491
64.0 BobWhite_c292_385
64.9 Excalibur_c7552_1959
66.3 BobWhite_c33540_69
67.2 Tdurum_contig28498_73
68.2 wmc0517b
68.6 wsnp_BE605194B_Ta_2_7
81.8 Tdurum_contig12525_769
82.2 Kukri_c345_644
83.1 Ku_c5789_663
84.0 wmc0311
84.5 wsnp_Ra_c39394_47110214
85.0 wsnp_Ex_rep_c101269_86663549
86.8 wsnp_Ku_c707_1465395
88.6 Tdurum_contig44876_1362
91.4 scm0002
QGY.asw-7B
QRGY.asw-7B
QRGY.asw-7B
QPY.asw-7B
QRPY.asw-7B


7D
0.0 RAC875_c31483_117
0.5 GENE.4461_104
5.5 BobWhite_c8415_728
6.0 BS00022449_51
6.4 BS00110124_51
6.9 gwm0635
8.7 Ra_c2573_3696
23.4 RAC875_c101180_108
36.4 Ra_c63534_581
36.5 RAC875_c54424_59
37.3 wsnp_Ex_c35_77935
40.1 tplb0024a09_742
51.2 barc0092
52.7 gwm295
58.2 BS00031820_51
66.1 D_GBF1XID02IP0NJ_181
75.4 BobWhite_rep_c57051_479
77.2 Ku_c884_1017
77.6 RAC875_c19328_198
79.0 RAC875_c10636_525
82.7 Kukri_c100613_331
86.4 RAC875_c53629_483
87.3 BS00051338_51
87.7 stm0001tcac
88.2 CAP8_rep_c9420_186
88.7 D_GBB4FNX01CLBM2_184
89.6 Ex_c8238_637
90.5 CAP7_rep_c9901_86
93.2 wsnp_BE497845D_Ta_1_1
96.0 IAAV4510
96.9 BS00022610_51
97.8 BS00062644_51
98.3 BS00070188_51
99.7 Ra_c10518_735
123.3 BS00067285_51
142.0 BobWhite_c48395_143
146.2 RAC875_c3826_2443
148.9 Ra_c74878_265
152.6 Ku_c48337_1131
153.0 Ku_c1234_733
153.5 wPt.0373
155.4 D_contig19288_196
159.0 BS00074366_51
166.0 BS00003945_51
166.4 Excalibur_c10102_911
QGY.asw-7D
QGY.asw-7D
QPY.asw-7D
XX
0.0 Excalibur_rep_c111629_239
0.5 GENE.2160_206
0.9 RFL_Contig1049_1473
1.4 Excalibur_rep_c69865_106
1.9 RAC875_rep_c117636_495
2.3 Tdurum_contig46252_1395
2.8 Ra_c10072_676
3.2 Tdurum_contig11509_373
3.7 RAC875_c95081_166

Chapter 6

Statement of Authorship

Title of Paper: Evaluation of nitrogen response in Australian genotypes under field and controlled conditions

Publication Status: Publication Style

Publication Details: Saba Mahjourimajd, Sanjiv Satjia, Julian Taylor, Haydn Kuchel, Peter Langridge, Mamoru Okamoto, 2015, Euphytica

Author Contributions

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

Saba Mahjourimajd Performed analysis on all samples, interpreted data and wrote manuscript.

Signature **Date** 17/12/14

Sanjiv Satjia Contributed to the field experiment

Signature **Date** 09/12/2014

Dr. Julian Taylor Analysed the data, contributed to the ideas and manuscript

Signature **Date** 10/12/2014

Dr. Haydn Kuchel Supervised development of work, helped in data interpretation and manuscript evaluation

Signature **Date** 15/12/14

Prof. Peter Langridge Supervised development of work, helped in data interpretation and manuscript evaluation

Signature **Date** 15/12/2014

Dr. Mamoru Okamoto Supervised development of work, helped in data interpretation and manuscript evaluation

Signature **Date** 15/12/2014

Evaluation of nitrogen response in Australian genotypes under field and controlled conditions

Saba Mahjourimajd[1], Sanjiv Satjia[1], Julian Taylor[2], Haydn Kuchel[3], Peter Langridge[1], Mamoru Okamoto[1]

[1]Australian Centre for Plant Functional Genomics (ACPFG), University of Adelaide, PMB1, Glen Osmond, SA 5064, Australia

[2]School of Agriculture, Food and Wine, Waite Research Institute, University of Adelaide, PMB 1, Glen Osmond, SA 5064, Australia

[3]Australian Grain Technologies, PMB1, Glen Osmond, SA 5064, Australia

Abstract

Hydroponic experiments were conducted to better understand the physiological aspect of nitrogen use efficiency (NUE) through the measurement of nitrogen (N) uptake-related traits under controlled conditions. Nitrate influx was measured at the high-affinity range by using ^{15}N in 3 week old wheat lines of a mapping population derived from a cross between RAC875 and Kukri. These lines were selected according to their yield performance at varying N levels in NUE-field trials in Australia conducted between 2011 and 2013. The aim of this experiment was to elucidate the physiological differences at an early developmental stage in N responsiveness between lines that showed a positive or negative response in the field trials. The results showed that there was no significant difference between the lines for the traits measured in the hydroponics experiments. There are two possible explanations for these results. Firstly, the lack of correlation between N responses at early vegetative stages and the final grain yield in the field. Alternatively different growth conditions, field versus controlled environment, could account for the differing responses. There is a need for further

experiments at a range of growth stages to investigate whether differences in nitrate uptake and N utilisation could explain the observed contrasting performances under field conditions.

Keywords: Nitrogen (N). Uptake capacity. Hydroponics experiments. Wheat, Selection

Authors:

Saba Mahjourimajd (saba.mahjourimajd@adelaide.edu.au)

Sanjiv Satjia (sanjiv.satjia@acpfg.com.au)

Haydn Kuchel (haydn.kuchel@ausgraintech.com.au)

Peter Langridge (peter.langridge@adelaide.edu.au)

Mamoru Okamoto (mamoru.okamoto@acpfg.com.au)

Corresponding author: Mamoru Okamoto,

e-mail: mamoru.okamoto@acpfg.com.au

Office phone: +61 8 83131499

Fax No: +61 8 8313 7102

Introduction

Wheat (*Triticum aestivum* L.) crops are not efficient in their use of nitrogen (N) supplied and particularly in the Mediterranean-type climate of southern Australia (Fillery and McInnes 1992). Therefore, N fertiliser application is costly and may have a negative impact on the environment due to the high rate of N loss through denitrification, leaching, run-off leading to environmental issues in water ways. To overcome this low N uptake efficiency, there may be an opportunity to enhance synchronisation of crop N demand and the N timing. Improving N uptake in plants should be possible by selecting genotypes showing high and fast root and shoot growth at the early stages of development (early vigour) (Liao et al. 2004) which would lead to improvements in nitrogen use efficiency (NUE).

Nitrogen use efficiency can be defined and assessed in different ways. Generally, NUE indicates the capability of plants to increase grain yield (GY) per unit of N supplied (Moll et al., 1982). Two main components are used to calculate NUE, N uptake efficiency (NupE, N uptake/N supplied), and N utilisation efficiency (NutE, GY/N uptake). To improve genotypes for better use of applied N, both physiological and molecular studies are useful to understand the role of N in growth and N response for the traits interest. In this study, NUE was assessed as the response by plants to varying N levels for several traits and used to study the behaviour of contrasting responsive plants toward N.

There are some studies showing the significance of root growth at the early stages of development in maize (Tuberosa et al. 2003) and for wheat (An et al. 2006) to GY in field conditions. However, little is known about the association between N uptake status during different developmental stages in wheat and GY at the final growth and grain filling stages. Typically, two uptake pathways control nutrient uptake in plants; a high-affinity transport system (HATS) and a low-affinity transport system (LATS) (Glass et al. 2002). High-affinity transport system uptake system becomes apparent when transporters are active at low external nitrate concentration (between 1 μM and 1 mM) while LATS is active when the concentration exceeds (between 200 to 500 μM).

The uptake pathway of N in plants is controlled by different genes, environmental factors and the interaction of these. The root system is the main part of the plant which affects the uptake rate and mechanism and is influenced by root distribution and growth, the activities of different N transporters and metabolic enzymes, N availability and growth conditions. Nitrogen use efficiency improvement relies on a good understanding of uptake component with integration of physiological and molecular approaches. Physiological techniques such as using isotopic forms of N (i.e. ^{13}N and ^{15}N) as a tracer are useful to monitor and measure N uptake by roots particularly at high concentrations and over short experimental periods (Glass 2003). Nitrogen isotope discrimination expressed as $\delta^{15}N$, can be affected by the combination of N supply and demand. The discrimination of heavier N isotope (^{15}N) against the lighter one (^{14}N) through a kinetic process of the fractionation of $\delta^{15}N$ makes it variable in root and shoot (Robinson 2001; Kalcsits and Guy 2013).

To confirm the differences in NUE and N response, two controlled-environment experiments were carried out using ten genotypes selected from the field trials which showed differing performances at different levels N application (Chapter 4). The aims of this study were 1) to examine the association between NUE field trials and N uptake capacity at the early growth stage of plants; 2) to study the uptake behaviour of contrasting responsive doubled haploid lines selected from the population studied in the field trials; and 3) to assess physiological aspect of N response.

Materials and methods

Plant materials

A population derived from a cross between RAC875 and Kukri (Fleury et al. 2010) was used to study N response of a sub-population comprised of 156 DH lines in a series of NUE field trials in Australia (Chapter 4). The ten highest-yielding and most N-responsive lines and the ten least responsive and lowest-yielding lines were ranked and identified in field experiments. These 20 lines were grown and evaluated at two field trials in South Australia in 2013 (GER

13 and TAR 13) (Table 3). The genetic map of the Kukri and RAC875 population was described by Bennett et al. (2012a). The female parent, RAC875 performed well under drought conditions and was relatively tolerant to water deficit while Kukri was rather drought sensitive.

Field experiments

The field trials were conducted in partial replication (except for the GER 13 and TAR 13 trials which were grown with three replicates) at different levels of N application, 2011 to 2013 (Table 1, Chapter 4- Table 3 in this chapter). Soil analyses were performed on subsamples of soil by CSBP Future Farm Analytical Laboratories (Bibra Lake, Australia). Standard region management practices were applied in all fields and years. N fertiliser was applied to the topsoil and on sowing date in South Australia and in the Western Australia. Grain yield (GY, kg ha^{-1}) was measured for all plots.

Hydroponic culture

Eight wheat DH lines were selected showing contrasting responses to N provision in the multi-environment NUE field trials in Australia (Chapter 4 for more details). These positive and negative N-response lines, four each, as well as the parental lines were assessed in hydroponic experiments, in 2013 and 2014, Exp. 1 and 2.

The hydroponic systems were similar to that previously described by Garnett et al. (2013). Seeds were germinated in moistened perlite for one week at room temperature. The seeds used were kept in fridge (at 4^{o} C) for a few days to ensure uniform germination. Two seedlings for each line with primary root and coleoptile (3-4 cm) were then transferred to the hydroponics system. The hydroponics system included big bins (two for each N concentration) and tubs (two connected to each bin). The lid on each tub had holes to keep the tubes (300 mm × 50 mm) in position. Plants were grown on mesh collar inside the tubes. The root system was in contact with the solution while being regularly aerated with compressed air (Garnett et al. 2013). The two experiments were conducted in different growth rooms although the environmental conditions were the same. In both experiments, plants were grown

hydroponically in a randomised complete-block design with four replicates in growth chambers under the following controlled conditions:

Light/dark periods: 16/8 h; temperature (light/dark): 21/15 °C, and relative humidity (light/dark): 50/60 %. Two seedlings of each line were placed in each tube and all the tubes replicated in four for each treatment were connected to the tank of 120 L nutrient solution with the following composition: 0.5 mM NO_3^--N, 1.0 mM S and 0.25 mM Ca for low N concentration (0.5 mM NO_3^--N) and 2.5 mM NO_3^--N, 0.5 mM S and 1.0 mM Ca for higher N concentration (2.5 mM NO_3^--N). Both concentrations contained 0.5 mM P, 1.05 mM K, 0.5 mM Mg, 0.05 mM Cl, 0.002 mM Mn, 0.002 mM Zn, 0.025 mM B and 0.1 mM Fe. Nutrient solutions were refreshed every week during the experiment and until harvest day which was after three weeks at the four-leaf stage. Fresh nutrient solution had a pH of 5.8. The pH in each tub was regularly checked and buffered with $CaCO_3$.

Nitrate influx measurement

Nitrate uptake capacity of the wheat plants was measured by short-term influx using ^{15}N labelled KNO_3 (Garnett et al. 2013). There was an electrical interruption of up to four hours in the growth room the day before harvest in Exp. 2. However, the plants were fresh and the experiment was continued.

In the same controlled growth conditions as during the growth period, the whole plants were transferred from the hydroponic growth tanks to the vessels containing the same growth solution. Then the roots were washed in the vessels in the solution without N, for 5 min. After the wash stage, the roots were immersed in the uptake vessels contained labelled solutions, 100 μM ^{15}N-labelled NO_3^- (10 atom% ^{15}N), to apply HATS uptake system, for 10 min. The roots were then dipped in non-labelled solutions for 2 min to rinse the root surface. All experiments were performed during a one hour window at mid-day to avoid diurnal effects on the uptake capacities. The plants were divided into shoots and roots and oven dried at 60°C for one week. The total dry weight (TDW) and the ratio of shoot to root (S/R DW).The finely ground plant samples in all treatments were weighed into tin capsules (3-4 mg each) and

analysed for δ^{15}N and N concentration by isotope ratio mass spectrometry (University of California Stable Isotope Facility, Davis, CA). δ^{15}N (%) is measured as (Handley and Raven 1992):

$$\delta^{15}N\ (\%) = \left(\frac{R_{sample} - R_{standard}}{R_{standard}}\right) 1000$$

Where R_{sample} is the $^{15}N/^{14}N$ isotope ratio of the sample and $R_{standard}$ is the isotope ratio of a known standard ($^{15}N/^{14}N$ ratio of air=0.00365). The natural abundance of ^{15}N of the N_2 in the air is constant (0.3663 atom % ^{15}N; (Junk and Svec 1958)). This constant value was used in all calculations of nitrate influx indicating uptake capacity and translocation of N to shoots in these experiments. Further measurements included N uptake in roots (RN) and shoots (SN), shoot to root N ratio (S/R N) and total N uptake (root + shoot) in plants.

Statistical analysis

The analysis of field data and selection of contrasting lines were described in Chapter 4. The data at GER 13 and TAR 13 and also general analysis of variance of the measured traits and parameters in hydroponics conditions were done using GenStat 15 (Payne 2009).

Results

There was significant genetic variation for GY in the population studied and genotypes responded differently to varying N levels across NUE field trials and treatments (Chapter 4, Table 2). The analysis of responsiveness BLUPs against efficiency data in a multi-environment analysis demonstrated significant G×N×E interaction for GY in the population (Chapter 4, Fig. 1).

We selected contrasting lines showing consistently positive or negative responses to N (Fig. 1). In comparison to the negative responsive lines, the positive responsive lines showed consistently enhanced GY and higher response to N with increasing amount of N fertiliser at the Pinery sites. Grain yield of the selected contrasting DH lines was analysed in NUE field trial at the GER 13 and TAR 13 sites in 2013. Genetic variation for average GY was

significant, although not very strong for the GER 13 trial but the effect of N treatment was significant at both sites. The effect of G×N was significant only at GER 13 indicating variable response to N treatments in this site (Table 4 and Fig. 2).

Hydroponics

The results of the hydroponics experiments using the contrasting N-responsive lines, and the parental lines, revealed that there was genetic variation for S/R DW, nitrate influx, translocation to shoot and S/R N in both experiments (Table 1). Nitrogen effect was significant for most of the traits in Exp. 2 but G×N was insignificant (Table 1). To better assess the positive and negative N-responsive lines, we separately analysed these lines excluding parents. According to the field results we expected to find difference for the uptake-related traits between the contrasting lines. However, the results showed little difference or variation among the contrasting lines for the measured traits (Table 2). Figs. 3 and 4 show root and shoot biomass for individual genotypes and indicate that there is no response to N concentrations for the traits in the two experiments (Table 1). However, the genotypes did differ significantly for S/R DW in these experiments (Fig. 5). The genotypes varying for nitrate influx and uptake capacity are depicted in Fig. 6 showing significant response to N in some lines. However, the contrasting lines demonstrated inconsistent results for variation and response to N of nitrate influx and uptake capacity in these experiments (Table 2).

Translocation into the shoot showed significant genetic variation among the DH and parental lines but the response to N treatments and G×N was different between the experiments (Table 1 and Fig. 7). However, there was no clear variation for this measurement between positive and negative lines (Table 2).

N uptake

The results of N uptake in positive and negative lines are presented in Fig. 8 to 10. There was a significant difference between the contrasting lines for N accumulation in root under controlled conditions (Table 2 and Fig. 8). Only in Exp. 2, genotypes showed higher N in root

and shoot samples at the higher level of N treatment (Figs. 8 and 9). In addition, S/R N uptake had no significant results in these experiments.

Discussion

Strong response to high N and better performance for GY for the positive responsive lines compared with negative lines in different environments is clear from the results shown in Fig. 1. The hydroponic experiments were carried out to study uptake capacity and physiological traits under different N concentrations. The insignificant differences for DW in this study were in line with the results by Garnett et al. (2013) who realised no difference in either root or shoot biomass of maize grown at different N concentrations (i.e. 0.5 and 2.5 mM N).

Nitrogen isotope discrimination can be affected by variation in N supply and demand. Therefore, fractionation of δ^{15}N can be due to isotopic discrimination changes in the root and the shoot (Robinson 2001; Kalcsits and Guy 2013). Coque et al. (2006) dissected the physiological and genetic variation of ^{15}N/^{14}N isotope ratio in maize plants under field and hydroponic conditions. They identified higher genetic variation in ^{15}N discrimination ability at low N concentration than that at high level. In addition, their results in hydroponics system showed that ^{15}N abundance at silking correlated negatively with the fresh root weight and glutamine synthetase (GS) activity in early of vegetative growth (Coque et al. 2006).

The results of nitrate influx (μmoles/gDW/hr) indicated that the contrasting lines responded significantly to N in Exp. 2. However, the insignificant effects of genotype (G) and N were unexpected. It should be highlighted that the electrical interruption in the hydroponic system might have caused some changes in the balance between N demand and supply altering the nitrate high-affinity transport system.

Stable N isotope, δ^{15}N and δ^{13}C was used to integrate the stress responses of barley plants during vegetative growth in a hydroponics system (Robinson et al. 2000). Robinson et al. (2000) showed that δ^{15}N varied in root and shoot under drought stress indicating S/R δ^{15}N is a good index for the stress response. There are few studies of N uptake under controlled

condition in wheat. Gioseffi et al. (2012) used N isotope composition to study the interaction between uptake of amino acids and inorganic N in wheat plants. The low ratio of ^{13}C and ^{15}N in shoots compared to roots indicated that C is lost more than N via respiration suggesting an interaction between the uptake of inorganic and organic N (Gioseffi et al. 2012). Liao et al. (2004) studied N uptake of a breeding line Vigour 18 along with four more Australian wheat cultivars and demonstrated that early vigour lines with bigger root and shoot biomass displayed increased nitrate reductase activity and N uptake compared to control lines. They described that the lines selected for greater early vigour showed larger root growth and shoot biomass and N uptake at tillering when grown in the field (Pang et al. 2013). The results in the present research did not support previous findings in terms of early vigour. There are some molecular studies showing co-localisation of genome regions controlling GY and its components in field trials with the traits interest such as root and shoot dry weight, and N uptake under controlled conditions (An et al. 2006; Laperche et al. 2006b). However, some studies demonstrated a mismatch and conflicting results when results from field and controlled conditions were compared (Robinson et al. 2000). Passioura (2010) explained that the unsuccessful scaling up in agricultural research between field and controlled conditions can be caused by the multi-genic nature of many key agronomic traits. Plant diseases and the constraints and interacting factors during growth under different conditions all contribute to performance under field versus controlled growth conditions.

We observed no significant treatment differences in Exp. 2 but significant differences for nitrate uptake in the two experiments. To understand these results we should take into account the age of the plants and the growth conditions. We might have missed the time point and growth stage needed to the trace the difference between the positive and negative lines selected using GY under field conditions.

Conclusion

The selected lines chosen for the response to N was confirmed for GY at varying N application in the new set of field trials at GER 13 and TAR 13. However, there was no observed association between field trials and hydroponics conditions for the genotypes studied at a vegetative stage of growth. Further work is needed to determine the optimal growth stage(s) to capture differences between the positive and negative N-responsive genotypes. Integration of physiological and genetic dissection for N response in a large number of genotypes at critical growth stages and under various environments could help identify the genes underlying N response and result in NUE and GY improvement.

References

Abe A, Menkir A, Moose SP, Adetimirin VO, Olaniyan AB (2013) Genetic variation for nitrogen-use efficiency among selected tropical maize hybrids differing in grain yield potential. Journal of Crop Improvement 27:31-52

Agrama H, Zakaria A, Said F, Tuinstra M (1999) Identification of quantitative trait loci for nitrogen use efficiency in maize. Mol Breed 5:187-195

An D, Su J, Liu Q, Zhu Y, Tong Y, Li J, Jing R, Li B, Li Z (2006) Mapping QTLs for nitrogen uptake in relation to the early growth of wheat (Triticum aestivum L.). Plant Soil 284:73-84

Angus J, Van Herwaarden A (2001) Increasing water use and water use efficiency in dryland wheat. Agron J 93:290-298

Asplund L, Bergkvist G, Weih M (2014) Proof of concept: nitrogen use efficiency of contrasting spring wheat varieties grown in greenhouse and field. Plant Soil 374:829-842

Bancal P (2009) Decorrelating source and sink determinism of nitrogen remobilization during grain filling in wheat. Ann Bot 103:1315-1324

Barbottin A, Lecomte C, Bouchard C, Jeuffroy M-H (2005) Nitrogen remobilization during grain filling in wheat. Crop Sci 45:1141-1150

Basso B, Cammarano D, Troccoli A, Chen D, Ritchie JT (2010) Long-term wheat response to nitrogen in a rainfed Mediterranean environment: Field data and simulation analysis. Eur J Agron 33:132-138

Bennett D, Izanloo A, Edwards J, Kuchel H, Chalmers K, Tester M, Reynolds M, Schnurbusch T, Langridge P (2012a) Identification of novel quantitative trait loci for days to ear emergence and flag leaf glaucousness in a bread wheat (Triticum aestivum L.) population adapted to southern Australian conditions. Theor Appl Genet 124:697-711

Bennett D, Izanloo A, Reynolds M, Kuchel H, Langridge P, Schnurbusch T (2012b) Genetic dissection of grain yield and physical grain quality in bread wheat (Triticum aestivum L.) under water-limited environments. Theor Appl Genet 125:255-271

Bertin P, Gallais A (2001) Genetic variation for nitrogen use efficiency in a set of recombinant inbred lines II - QTL detection and coincidences. Maydica 46:53-68

Blackmer T, Schepers J (1994) Techniques for monitoring crop nitrogen status in corn. Commun Soil Sci Plant Anal 25:1791-1800

Blum A (1988) Plant breeding for stress environments.

Bogard M, Allard V, Brancourt-Hulmel M, Heumez E, Machet JM, Jeuffroy MH, Gate P, Martre P, Le Gouis J (2010) Deviation from the grain protein concentration–grain yield negative relationship is highly correlated to post-anthesis N uptake in winter wheat. J Exp Bot 61:4303-4312

Bogard M, Jourdan M, Allard V, Martre P, Perretant MR, Ravel C, Heumez E, Orford S, Snape J, Griffiths S (2011) Anthesis date mainly explained correlations between post-anthesis leaf senescence, grain yield, and grain protein concentration in a winter wheat population segregating for flowering time QTLs. J Exp Bot 62:3621-3636

Bordes J, Ravel C, Jaubertie J, Duperrier B, Gardet O, Heumez E, Pissavy A, Charmet G, Le Gouis J, Balfourier F (2013) Genomic regions associated with the nitrogen limitation response revealed in a global wheat core collection. Theor Appl Genet 126:805-822

Brancourt-Hulmel M, Heumez E, Pluchard P, Beghin D, Depatureaux C, Giraud A, Le Gouis J (2005) Indirect versus direct selection of winter wheat for low-input or high-input levels. Crop Sci 45:1427-1431
Brauer EK, Shelp BJ (2010) Nitrogen use efficiency: re-consideration of the bioengineering approach. Botany 88:103-109
Brennan J, Hackett R, McCabe T, Grant J, Fortune R, Forristal P (2014) The effect of tillage system and residue management on grain yield and nitrogen use efficiency in winter wheat in a cool Atlantic climate. Eur J Agron 54:61-69
Chantret N, Salse J, Sabot F, Rahman S, Bellec A, Laubin B, Dubois I, Dossat C, Sourdille P, Joudrier P, Gautier MF, Cattolico L, Beckert M, Aubourg S, Weissenbach J, Caboche M, Bernard M, Leroy P, Chalhoub B (2005) Molecular basis of evolutionary events that shaped the hardness locus in diploid and polyploid wheat species (Triticum and aegilops). Plant Cell 17:1033-1045
Chardon F, Noël V, Masclaux-Daubresse C (2012) Exploring NUE in crops and in Arabidopsis ideotypes to improve yield and seed quality. J Exp Bot 62:2131-2142
Chardon F, Virlon B, Moreau L, Falque M, Joets J, Decousset L, Murigneux A, Charcosset A (2004) Genetic architecture of flowering time in maize as inferred from quantitative trait loci meta-analysis and synteny conservation with the rice genome. Genetics 168:2169-2185
Charmet G, Robert N, Branlard G, Linossier L, Martre P, Triboï E (2005) Genetic analysis of dry matter and nitrogen accumulation and protein composition in wheat kernels. Theor Appl Genet 111:540-550
Chen C, Westcott M, Neill K, Wichman D, Knox M (2004) Row configuration and nitrogen application for barley–pea intercropping in Montana. Agron J 96:1730-1738
Chen ZJ (2007) Genetic and epigenetic mechanisms for gene expression and phenotypic variation in plant polyploids. Annu Rev Plant Biol 58:377
Cho Y, Jiang W, Chin J, Piao Z, Cho Y, McCouch SR, Koh H (2007) Identification of QTLs associated with physiological nitrogen use efficiency in rice. Mol Cells 23:72-79
Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138:963-971
Collard B, Jahufer M, Brouwer J, Pang E (2005) An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. Euphytica 142:169-196
Cooper M, DeLacy I, Basford K (1996) Relationships among analytical methods used to analyse genotypic adaptation in multi-environment trials. Plant adaptation and crop improvement, 193-224. CAB Int., Wallingford, UK
Coque M, Bertin P, Hirel B, Gallais A (2006) Genetic variation and QTLs for< sup> 15</sup> N natural abundance in a set of maize recombinant inbred lines. Field Crops Res 97:310-321
Coque M, Gallais A (2006) Genomic regions involved in response to grain yield selection at high and low nitrogen fertilization in maize. Theor Appl Genet 112:1205-1220
Coque M, Martin A, Veyrieras J, Hirel B, Gallais A (2008) Genetic variation for N-remobilization and postsilking N-uptake in a set of maize recombinant inbred lines. 3. QTL detection and coincidences. Theor Appl Genet 117:729-747
Cormier F, Faure S, Dubreuil P, Heumez E, Beauchêne K, Lafarge S, Praud S, Le Gouis J (2013) A multi-environmental study of recent breeding progress on nitrogen use efficiency in wheat (Triticum aestivum L.). Theor Appl Genet 126:3035-3048
Cormier F, Le Gouis J, Dubreuil P, Lafarge S, Praud S (2014) A genome-wide identification of chromosomal regions determining nitrogen use efficiency components in wheat (Triticum aestivum L.). Theor Appl Genet:1-15
Cox MC, Qualset CO, Rains DW (1985) Genetic-variation for nitrogen assimilation and translocation in wheat .2. Nitrogen assimilation in relation to grain-yield and protein. Crop Sci 25:435-440
Craswell E, Godwin D (1984) The efficiency of nitrogen fertilizers applied to cereals grown in different climates. Advances in plant nutrition 1:1-55
Crews TE, Peoples MB (2005) Can the synchrony of nitrogen supply and crop demand be improved in legume and fertilizer-based agroecosystems? A review. Nutrient Cycling in Agroecosystems 72:101-120
Dhugga KS, Waines J (1989) Analysis of nitrogen accumulation and use in bread and durum wheat. Crop Sci 29:1232-1239
Elouafi I, Nachit M, Elsaleh A, Asbati A, Mather D (2000) QTL-mapping of genomic regions controlling gluten strength in durum (Triticum turgidum L. var. durum). Durum wheat improvement in the Mediterranean region: New challenges International Centre for Advanced Mediterranean Agronomic Studies, Zaragoza, Spain 40:363-371
Ercoli L, Lulli L, Mariotti M, Masoni A, Arduini I (2008) Post-anthesis dry matter and nitrogen dynamics in durum wheat as affected by nitrogen supply and soil water availability. Eur J Agron 28:138-147
Fageria N, Baligar V (2005) Enhancing nitrogen use efficiency in crop plants. Adv Agron 88:97-185
FAO (2011) Current world fertilizer trends and outlook to 2015. ftp://ftp.fao.org/ag/agp/docs/cwfto15.pdf.
FAO (2012). www.fao.org.
Fillery I, McInnes K (1992) Components of the fertiliser nitrogen balance for wheat production on duplex soils. Anim Prod Sci 32:887-899

Fleury D, Jefferies S, Kuchel H, Langridge P (2010) Genetic and genomic tools to improve drought tolerance in wheat. J Exp Bot 61:3211-3222
Flowers M, Tarleton B, Meijer A, Heiniger R, Weisz R (2003) Field validation of a remote sensing technique for early nitrogen application decisions in wheat. Agron J 95:167-176
Fontaine J-X, Ravel C, Pageau K, Heumez E, Dubois F, Hirel B, Le Gouis J (2009) A quantitative genetic study for elucidating the contribution of glutamine synthetase, glutamate dehydrogenase and other nitrogen-related physiological traits to the agronomic performance of common wheat. Theor Appl Genet 119:645-662
Gallais A, Coque M (2005) Genetic variation and selection for nitrogen use efficiency in maize: a synthesis. Maydica 50:531-547
Gallais A, Coque M, Quilléré I, Prioul JL, Hirel B (2006) Modelling postsilking nitrogen fluxes in maize (Zea mays) using 15N-labelling field experiments. New Phytol 172:696-707
Gallais A, Hirel B (2004) An approach to the genetics of nitrogen use efficiency in maize. J Exp Bot 55:295-306
Gan Y, Miller P, McConkey B, Zentner R, Stevenson F, McDonald C (2003) Influence of diverse cropping sequences on durum wheat yield and protein in the semiarid Northern Great Plains. Agron J 95:245-252
Garnett T, Conn V, Plett D, Conn S, Zanghellini J, Mackenzie N, Enju A, Francis K, Holtham L, Roessner U (2013) The response of the maize nitrate transport system to nitrogen demand and supply across the lifecycle. New Phytol 198:82-94
Gasser J, Thorburn M (1972) The growth, composition and nutrient uptake of spring wheat. J Agric Sci 78:393-404
Gastal F, Lemaire G (2002) N uptake and distribution in crops: an agronomical and ecophysiological perspective. J Exp Bot 53:789-799
Geiger HH (2009) Agronomic Traits and Maize Modifications: Nitrogen Use Efficiency. Handbook of Maize: Its Biology:405-417
Gioseffi E, Neergaard Ad, Schjørring JK (2012) Interactions between uptake of amino acids and inorganic nitrogen in wheat plants. Biogeosciences 9:1509-1518
Glass AD, Britto DT, Kaiser BN, Kinghorn JR, Kronzucker HJ, Kumar A, Okamoto M, Rawat S, Siddiqi M, Unkles SE (2002) The regulation of nitrate and ammonium transport systems in plants. J Exp Bot 53:855-864
Glass ADM (2003) Nitrogen use efficiency of crop plants: Physiological constraints upon nitrogen absorption. CRC Crit Rev Plant Sci 22:453-470
Goffinet B, Gerber S (2000) Quantitative trait loci: a meta-analysis. Genetics 155:463-473
Good AG, Johnson SJ, De Pauw M, Carroll RT, Savidov N, Vidmar J, Lu Z, Taylor G, Stroeher V (2007) Engineering nitrogen use efficiency with alanine aminotransferase. Botany 85:252-262
Good AG, Shrawat AK, Muench DG (2004) Can less yield more? Is reducing nutrient input into the environment compatible with maintaining crop production? Trends Plant Sci 9:597-605
Gouis J, Pluchard P (1996) Genetic variation for nitrogen use efficiency in winter wheat (Triticum aestivum L.). Euphytica 92:221-224
Goulding K (2004) Pathways and losses of fertilizer nitrogen at different scales. Agriculture and the Nitrogen Cycle:209-219
Greenwood DJ, Karpinets TV, Zhang K, Bosh-Serra A, Boldrini A, Karawulova L (2008) A unifying concept for the dependence of whole-crop n : P ratio on biomass: Theory and experiment. Ann Bot 102:967-977
Gregory P, Crawford D, McGowan M (1979) Nutrient relations of winter wheat. Accumulation and distribution of Na, K, Ca, Mg, P, S, and NJ Agric Sci Camb 93:485-494
Groos C, Robert N, Bervas E, Charmet G (2003) Genetic analysis of grain protein-content, grain yield and thousand-kernel weight in bread wheat. Theor Appl Genet 106:1032-1040
Guarda G, Padovan S, Delogu G (2004) Grain yield, nitrogen-use efficiency and baking quality of old and modern Italian bread-wheat cultivars grown at different nitrogen levels. Eur J Agron 21:181-192
Gulmezoglu N, Aytac Z (2010) Response of grain and protein yields of triticale varieties at different levels of applied nitrogen fertilizer. Afr J Agric Res 5:2563-2569
Guo Y, Kong F-m, Xu Y-f, Zhao Y, Liang X, Wang Y-y, An D-g, Li S-s (2012) QTL mapping for seedling traits in wheat grown under varying concentrations of N, P and K nutrients. Theor Appl Genet 124:851-865
Habash D, Massiah A, Rong H, Wallsgrove R, Leigh RA (2001) The role of cytosolic glutamine synthetase in wheat. Ann Appl Biol 138:83-89
Habash DZ, Bernard S, Schondelmaier J, Weyen J, Quarrie SA (2007) The genetics of nitrogen use in hexaploid wheat: N utilisation, development and yield. Theor Appl Genet 114:403-419
Handley L, Raven JA (1992) The use of natural abundance of nitrogen isotopes in plant physiology and ecology. Plant Cell Environ 15:965-985
Hao QN, Zhou XA, Ai HS, Wang C, Zhou R, Chen SL (2011) Identification of genes associated with nitrogen-use efficiency by genome-wide transcriptional analysis of two soybean genotypes. BMC Genomics 12:525
Harper L, Langdale G, Giddens J (1987) Nitrogen cycling in a wheat crop: soil, plant, and aerial nitrogen transport. Agron J 79:965-973

Harrison R, Webb J (2001) A review of the effect of N fertilizer type on gaseous emissions. Adv Agron 73:65-108
Hibberd JM, Sheehy JE, Langdale JA (2008) Using C-4 photosynthesis to increase the yield of rice - rationale and feasibility. Curr Opin Plant Biol 11:228-231
Hirel B, Bertin P, Quilleré I, Bourdoncle W, Attagnant C, Dellay C, Gouy A, Cadiou S, Retailliau C, Falque M (2001) Towards a better understanding of the genetic and physiological basis for nitrogen use efficiency in maize. Plant Physiol 125:1258-1270
Hirel B, Le Gouis J, Ney B, Gallais A (2007) The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. J Exp Bot 58:2369-2387
Hirel B, Lea PJ (2001) Ammonia assimilation. Plant Nitrogen.
Hodge A, Robinson D, Fitter A (2000) Are microorganisms more effective than plants at competing for nitrogen? Trends Plant Sci 5:304-308
Ikram S, Chardon F (2010) Plant quantitative traits. eLS
Joppa L, Du C, HarT GE, Hareland GA (1997) Mapping gene (s) for grain protein in tetraploid wheat (Triticum turgidum L.) using a population of recombinant inbred chromosome lines. Crop Sci 37:1586-1589
Junk G, Svec HJ (1958) The absolute abundance of the nitrogen isotopes in the atmosphere and compressed gas from various sources. Geochimica et Cosmochimica Acta 14:234-243
Justes E, Mary B, Meynard JM, Machet JM, Thelierhuche L (1994) Determination of a critical nitrogen dilution curve for winter-wheat crops. Ann Bot 74:397-407
Kalcsits LA, Guy RD (2013) Whole-plant and organ-level nitrogen isotope discrimination indicates modification of partitioning of assimilation, fluxes and allocation of nitrogen in knockout lines of Arabidopsis thaliana. Physiol Plant 149:249-259
Kanampiu FK, Raun WR, Johnson GV (1997) Effect of nitrogen rate on plant nitrogen loss in winter wheat varieties 1. J Plant Nutr 20:389-404
Kichey T, Hirel B, Heumez E, Dubois F, Le Gouis J (2007) In winter wheat (Triticum aestivum L.), post-anthesis nitrogen uptake and remobilisation to the grain correlates with agronomic traits and nitrogen physiological markers. Field Crops Res 102:22-32
Kjaer B, Jensen J (1996) Quantitative trait loci for grain yield and yield components in a cross between a six-rowed and a two-rowed barley. Euphytica 90:39-48
Laperche A, Brancourt-Hulmel M, Heumez E, Gardet O, Gouis JL (2006a) Estimation of genetic parameters of a DH wheat population grown at different N stress levels characterized by probe genotypes. Theor Appl Genet 112:797-807
Laperche A, Brancourt-Hulmel M, Heumez E, Gardet O, Hanocq E, Devienne-Barret F, Le Gouis J (2007) Using genotype× nitrogen interaction variables to evaluate the QTL involved in wheat tolerance to nitrogen constraints. Theor Appl Genet 115:399-415
Laperche A, Devienne-Barret F, Maury O, Le Gouis J, Ney B (2006b) A simplified conceptual model of carbon/nitrogen functioning for QTL analysis of winter wheat adaptation to nitrogen deficiency. Theor Appl Genet 113:1131-1146
Le Gouis J (2011) Genetic Improvement of Nutrient Use Efficiency in Wheat" in Molecular basis of nutrient use efficiency incrops First edn. John Wiley, Oxford
Le Gouis J, Béghin D, Heumez E, Pluchard P (2000) Genetic differences for nitrogen uptake and nitrogen utilisation efficiencies in winter wheat. Eur J Agron 12:163-173
Lea PJ, Azevedo RA (2007) Nitrogen use efficiency. 2. Amino acid metabolism. Ann Appl Biol 151:269-275
Lemaire G, Gastal F (2009) Quantifying crop responses to nitrogen deficiency and avenues to improve nitrogen-use efficiency. Crop physiology: applications for genetic improvement and agronomy Academic Press, San Diego:171–211
Li J, Zhang Z, Li Y, Wang Q, Zhou Y (2011) QTL consistency and meta-analysis for grain yield components in three generations in maize. Theor Appl Genet 122:771-782
Lian X, Xing Y, Yan H, Xu C, Li X, Zhang Q (2005) QTLs for low nitrogen tolerance at seedling stage identified using a recombinant inbred line population derived from an elite rice hybrid. Theor Appl Genet 112:85-96
Liao MT, Fillery IRP, Palta JA (2004) Early vigorous growth is a major factor influencing nitrogen uptake in wheat. Funct Plant Biol 31:121-129
López-Bellido L, López-Garrido F, Fernández E, Fuentes M, Castillo J (1996) Long-term tillage, crop rotation, and nitrogen fertilizer effects on wheat yield under rainfed Mediterranean conditions. Agron J 88:783-791
Lopez-Bellido R, Shepherd C, Barraclough P (2004) Predicting post-anthesis N requirements of bread wheat with a Minolta SPAD meter. Eur J Agron 20:313-320
Loudet O, Chaillou S, Merigout P, Talbotec J, Daniel-Vedele F (2003) Quantitative trait loci analysis of nitrogen use efficiency in Arabidopsis. Plant Physiol 131:345-358
MacDonald WN, Blom TJ, Tsujita MJ, Shelp BJ (2013) Review: Improving nitrogen use efficiency of potted chrysanthemum: Strategies and benefits. Can J Plant Sci 93:1009-1016

MacKown CT, Carver BF (2005) Fall forage biomass and nitrogen composition of winter wheat populations selected from grain-only and dual-purpose environments. Crop Sci 45:322-328
Man H, Boriel R, El-Khatib R, Kirby EG (2005) Characterization of transgenic poplar with ectopic expression of pine cytosolic glutamine synthetase under conditions of varying nitrogen availability. New Phytol 167:31-39
Masclaux-Daubresse C, Daniel-Vedele F, Dechorgnat J, Chardon F, Gaufichon L, Suzuki A (2010) Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture. Ann Bot 105:1141-1157
Masclaux-Daubresse C, Reisdorf-Cren M, Orsel M (2008) Leaf nitrogen remobilisation for plant development and grain filling. Plant Biol 10:23-36
McDonald G (1992) Effects of nitrogenous fertilizer on the growth, grain yield and grain protein concentration of wheat. Aust J Agric Res 43:949-967
McIntosh R, Yamazaki Y, Devos K, Dubcovsky J, Rogers W, Appels R Catalogue of gene symbols for wheat. In: 10th International Wheat Genetics Symposium. Paestum (Italie), 2003.
Merah O (2001) Potential importance of water status traits for durum wheat improvement under Mediterranean conditions. J Agric Sci 137:139-145
Mickelson S, See D, Meyer FD, Garner JP, Foster CR, Blake TK, Fischer AM (2003) Mapping of QTL associated with nitrogen storage and remobilization in barley (Hordeum vulgare L.) leaves. J Exp Bot 54:801-812
Mizuta K, Matsumoto T, Hatate Y, Nishihara K, Nakanishi T (2004) Removal of nitrate-nitrogen from drinking water using bamboo powder charcoal. Bioresour Technol 95:255-257
Moll RH, Kamprath EJ, Jackson WA (1982) Analysis and interpretation of factors which contribute to efficiency of nitrogen-utilization. Agron J 74:562-564
Monaghan JM, Snape JW, Chojecki AJS, Kettlewell PS (2001) The use of grain protein deviation for identifying wheat cultivars with high grain protein concentration and yield. Euphytica 122:309-317
Obara M, Sato T, Sasaki S, Kashiba K, Nagano A, Nakamura I, Ebitani T, Yano M, Yamaya T (2004) Identification and characterization of a QTL on chromosome 2 for cytosolic glutamine synthetase content and panicle number in rice. Theor Appl Genet 110:1-11
Ortiz-Monasterio R, Sayre K, Rajaram S, McMahon M (1997) Genetic progress in wheat yield and nitrogen use efficiency under four nitrogen rates. Crop Sci 37:898-904
Oury F-X, Godin C (2007) Yield and grain protein concentration in bread wheat: how to use the negative relationship between the two characters to identify favourable genotypes? Euphytica 157:45-57
Pang J, Palta JA, Rebetzke GJ, Milroy SP (2013) Wheat genotypes with high early vigour accumulate more nitrogen and have higher photosynthetic nitrogen use efficiency during early growth. Funct Plant Biol 41:215-222
Passioura J (2010) Scaling up: the essence of effective agricultural research. Funct Plant Biol 37:585-591
Pathak RR, Ahmad A, Lochab S, Raghuram N (2008) Molecular physiology of plant nitrogen use efficiency and biotechnological options for its enhancement. Curr Sci 94:1394-1403
Payne RW (2009) GenStat, Wiley Interdisciplinary Reviews. Comput Stat 1:255-258
Pedersen A, Zhang K, Thorup-Kristensen K, Jensen LS (2010) Modelling diverse root density dynamics and deep nitrogen uptake-A simple approach. Plant Soil 326:493-510
Peng Y, Yu P, Li X, Li C (2013) Determination of the critical soil mineral nitrogen concentration for maximizing maize grain yield. Plant soil 372:41-51
Peoples MB, Boyer EW, Goulding KWT, Heffer P, Ochwoh VA, Vanlauwe B, Wood S, Yagi K, Van Cleemput O (2004) Pathways of nitrogen loss and their impacts on human health and the environment. Agriculture and the nitrogen cycle: assessing the impact of fertilizer use on food production and the environment.
Pestsova E, Ganal M, Röder M (2000) Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. Genome 43:689-697
Peterson C, Graybosch R, Baenziger P, Grombacher A (1992) Genotype and environment effects on quality characteristics of hard red winter wheat. Crop Sci 32:98-103
Podlich D, Cooper M, Basford K, Geiger H (1999) Computer simulation of a selection strategy to accommodate genotype environment interactions in a wheat recurrent selection programme. Plant Breeding 118:17-28
Qi Z, Wu Q, Han X, Sun Y, Du X, Liu C, Jiang H, Hu G, Chen Q (2011) Soybean oil content QTL mapping and integrating with meta-analysis method for mining genes. Euphytica 179:499-514
Quarrie S, Steed A, Calestani C, Semikhodskii A, Lebreton C, Chinoy C, Steele N, Pljevljakusić D, Waterman E, Weyen J (2005) A high-density genetic map of hexaploid wheat (Triticum aestivum L.) from the cross Chinese Spring× SQ1 and its use to compare QTLs for grain yield across a range of environments. Theor Appl Genet 110:865-880
Quraishi UM, Abrouk M, Murat F, Pont C, Foucrier S, Desmaizieres G, Confolent C, Rivière N, Charmet G, Paux E (2011) Cross-genome map based dissection of a nitrogen use efficiency ortho-metaQTL in bread wheat unravels concerted cereal genome evolution. Plant J 65:745-756
Rafalski A (2002) Applications of single nucleotide polymorphisms in crop genetics. Curr Opin Plant Biol 5:94-100

Raun WR, Johnson GV (1999) Improving nitrogen use efficiency for cereal production. Agron J 91:357-363
Reynolds M, Foulkes MJ, Slafer GA, Berry P, Parry MAJ, Snape JW, Angus WJ (2009) Raising yield potential in wheat. J Exp Bot 60:1899-1918
Robertson GP, Vitousek PM (2009) Nitrogen in agriculture: Balancing the cost of an essential resource. Annu Rev Environ Resour 34:97-125
Robinson D (2001) delta N-15 as an integrator of the nitrogen cycle. Trends Ecol Evol 16:153-162
Robinson D, Handley L, Scrimgeour C, Gordon D, Forster B, Ellis R (2000) Using stable isotope natural abundances (δ15N and δ13C) to integrate the stress responses of wild barley (Hordeum spontaneum C. Koch.) genotypes. J Exp Bot 51:41-50
Rothstein SJ (2007) Returning to our roots: making plant biology research relevant to future challenges in agriculture. Plant Cell 19:2695-2699
Sadras VO, Lawson C, Hooper P, McDonald GK (2012) Contribution of summer rainfall and nitrogen to the yield and water use efficiency of wheat in Mediterranean-type environments of South Australia. Eur J Agron 36:41-54
Serrano L, Filella I, Penuelas J (2000) Remote sensing of biomass and yield of winter wheat under different nitrogen supplies. Crop Sci 40:723-731
Shi J, Li R, Qiu D, Jiang C, Long Y, Morgan C, Bancroft I, Zhao J, Meng J (2009) Unraveling the complex trait of crop yield with quantitative trait loci mapping in Brassica napus. Genetics 182:851-861
Shrawat AK, Carroll RT, DePauw M, Taylor GJ, Good AG (2008) Genetic engineering of improved nitrogen use efficiency in rice by the tissue-specific expression of alanine aminotransferase. Plant Biotechnol J 6:722-732
Søgaard R, Alsterfjord M, MacAulay N, Zeuthen T (2009) Ammonium ion transport by the AMT/Rh homolog TaAMT1; 1 is stimulated by acidic pH. Pflugers Arch 458:733-743
Sosulski FW, Imafidon GI (1990) Amino acid composition and nitrogen-to-protein conversion factors for animal and plant foods. J Agric Food Chem 38:1351-1356
Svoboda P, Haberle J (2006) The effect of nitrogen fertilization on root distribution of winter wheat. Plant Soil Environ 52:308-313
Sylvester-Bradley R, Kindred DR (2009) Analysing nitrogen responses of cereals to prioritize routes to the improvement of nitrogen use efficiency. J Exp Bot 60:1939-1951
Tindall TA, Stark JC, Brooks RH (1995) Irrigated spring wheat response to topdressed nitrogen as predicted by flag leaf nitrogen concentration. J Prod Agric 8:46-52
Tuberosa R, Salvi S, Sanguineti MC, Maccaferri M, Giuliani S, Landi P (2003) Searching for quantitative trait loci controlling root traits in maize: a critical appraisal. In: Roots: The Dynamic Interface between Plants and the Earth. Springer, pp 35-54
Tucker H (2000) Hormones, mammary growth, and lactation: a 41-year perspective. J Dairy Sci 83:874-884
Ulas A, Behrens T, Wiesler F, Horst WJ (2013) Does genotypic variation in nitrogen remobilisation efficiency contribute to nitrogen efficiency of winter oilseed-rape cultivars (Brassica napus L.)? Plant soil 371:463-471
Uribelarrea M, Crafts-Brandner SJ, Below FE (2009) Physiological N response of field-grown maize hybrids (Zea mays L.) with divergent yield potential and grain protein concentration. Plant soil 316:151-160
Vansanford DA, Mackown CT (1986) Variation in nitrogen use efficiency among soft red winter-wheat genotypes. Theor Appl Genet 72:158-163
Voorrips R (2002) MapChart: software for the graphical presentation of linkage maps and QTLs. J Hered 93:77-78
Wang D, Xu Z, Zhao J, Wang Y, Yu Z (2011) Excessive nitrogen application decreases grain yield and increases nitrogen loss in a wheat–soil system. Acta Agriculturae Scandinavica, Section B-Soil & Plant Science 61:681-692
Wang G, Bronson KF, Thorp KR, Mon J, Badaruddin M (2014) Multiple Leaf Measurements Improve Effectiveness of Chlorophyll Meter for Durum Wheat Nitrogen Management. Crop Sci 54:817-826
Wang S, Basten C, Zeng Z (2007) Windows QTL cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC
Xu G, Fan X, Miller AJ (2012) Plant nitrogen assimilation and use efficiency. Annu Rev Plant Biol 63:153-182
Xu Y, Wang R, Tong Y, Zhao H, Xie Q, Liu D, Zhang A, Li B, Xu H, An D (2014) Mapping QTLs for yield and nitrogen-related traits in wheat: influence of nitrogen and phosphorus fertilization on QTL expression. Theor Appl Genet 127:59-72

Table 1 The significance (*P* - value) of genotype (G), N treatment (N) and their interactions of all genotypes (included parental lines) for physiological traits in hydroponics experiments, 2013-2014

	Exp. 1- 2013									
Factors	RDW	SDW	TDW	S/RDW	Nitrate influx	Translocation to shoot	RN	SN	Total N uptake	S/RN
G	n.s	0.039	n.s	<0.001	<0.001	<0.001	0.034	n.s	n.s	<0.001
N	n.s	n.s	n.s	n.s	<0.001	<0.001	<0.001	<0.001	<0.001	n.s
G×N	n.s	n.s	n.s	n.s	<0.001	n.s	n.s	n.s	n.s	n.s
	Exp. 2- 2014									
Factors	RDW	SDW	TDW	S/RDW	N influx	Translocation to shoot	RN	SN	Total N uptake	S/RN
G	n.s	n.s	n.s	0.019	<0.001	<0.001	n.s	n.s	n.s	0.030
N	0.033	<0.001	<0.001	<0.001	n.s	<0.001	n.s	n.s	<0.001	0.007
G×N	n.s	n.s	n.s	0.042	<0.001	<0.001	n.s	n.s	n.s	0.034

Not significant, n.s at $P > 0.05$; 5% significant at $P < 0.05$; 1% significant at $P < 0.01$

Table 2 The significance (*P* - value) of genotype (G), N treatment (N) and their interactions of positive lines with negative lines for physiological traits in hydroponics experiments, 2013-2014

	Exp. 1- 2013									
Factors	RDW	SDW	TDW	S/RDW	Nitrate influx	Translocation to shoot	RN	SN	Total N uptake	S/RN
G	n.s	n.s	n.s	n.s	<0.001	n.s	0.005	n.s	n.s	0.005
N	n.s	0.047	n.s	n.s	<0.001	<0.001	0.004	<0.001	<0.001	n.s
G×N	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
	Exp. 2- 2014									
Factors	RDW	SDW	TDW	S/RDW	N influx	Translocation to shoot	RN	SN	Total N uptake	S/RN
G	n.s	0.045	n.s	n.s	n.s	n.s	0.033	0.006	0.006	n.s
N	<0.001	<0.001	<0.001	<0.001	n.s	<0.001	<0.001	<0.001	<0.001	n.s
G×N	n.s	n.s	n.s	n.s	<0.001	<0.001	0.006	n.s	n.s	n.s

Not significant, n.s at $P > 0.05$; 5% significant at $P < 0.05$; 1% significant at $P < 0.01$

Table 3 The latitude (Lat ° S), longitude (Lon ° E), elevation above sea level (Elv, m), growth season total rainfall (mm), growth season number of hot days with temperature higher than 30 °C, soil characteristics at top 10 cm depth of soil before fertilisation, nitrogen (N) fertiliser levels and average grain yield (GY, kg ha^{-1}) in NUE field trials of Australian sites in 2013

Site	Year	Abbreviation	Lat (° S)	Lon (° E)	Elv (m)	Total rain (mm)	Hot day (d)	Soil texture	pH level ($CaCl_2$)	pH level (H_2O)	NH_4^+ nitrogen (mg kg^{-1})	NO_3^- nitrogen (mg kg^{-1})	N fertiliser levels (kg ha^{-1})	Average GY (kg ha^{-1})
GERANIUM	2013	GER 13	35.8	140.1	72	208	13	Loamy-sand	8.2	9	0.6	32.5	0, 75, 150	2190
TARLEE	2013	TAR 13	34.2	138.7	192	284	14	Clay-Loam	4.4	5.3	0.3	17.2	0, 75, 150	4356

Table 4 The significance (*P* - value) of genotype (G), nitrogen treatment (N) and their interactions of selected wheat lines on grain yield (GY, kg ha^{-1}) in nitrogen use efficiency filed trials in Australia, 2013

Factors	Site	
	GER 13	TAR 13
G	0.019	n.s
N	<0.001	<0.001
G×N	0.032	n.s

Not significant, n.s at $P > 0.05$; 5% significant at $P < 0.05$; 1% significant at $P < 0.01$

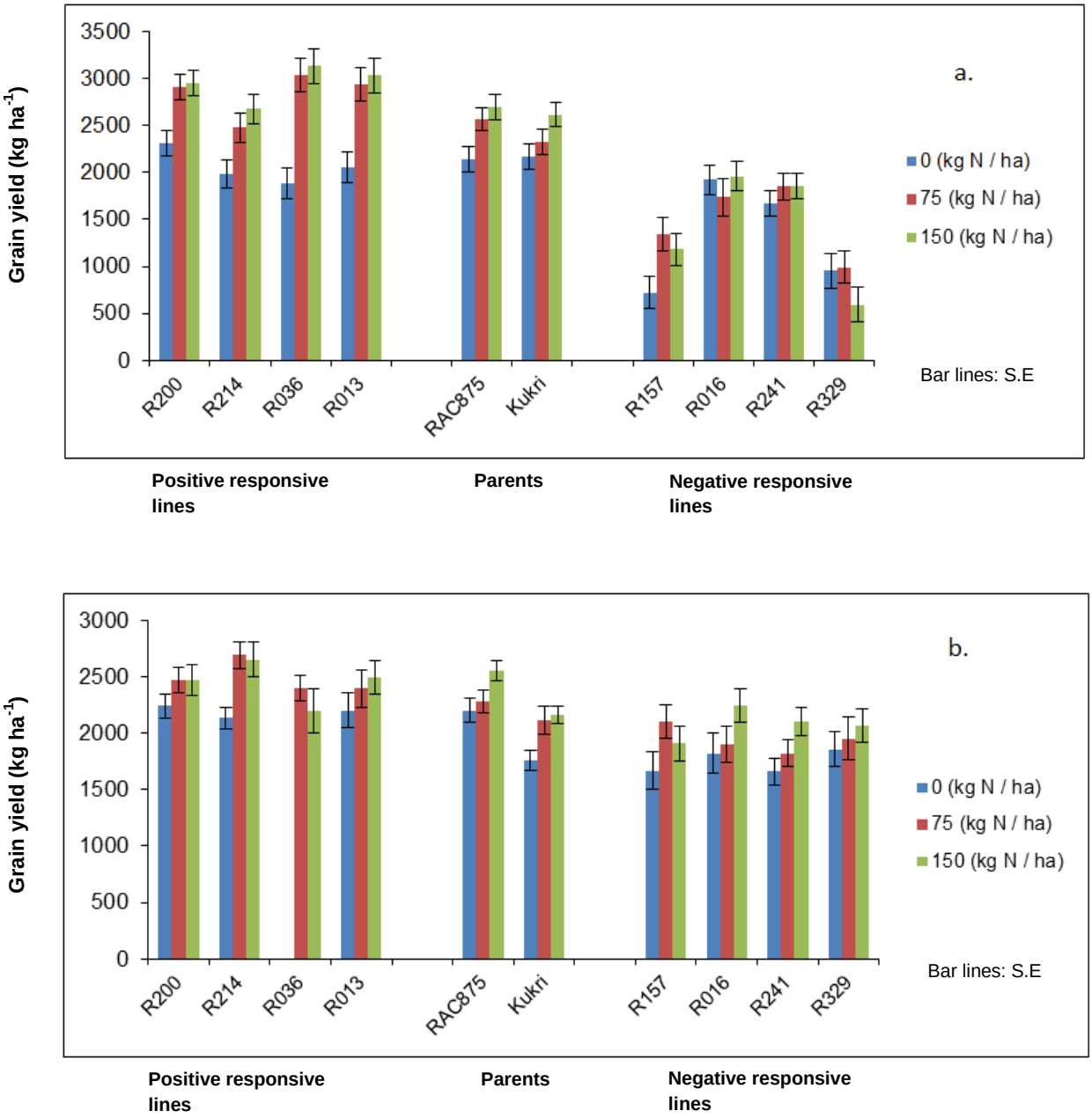


Fig. 1 Grain yield (kg ha^{-1}) of selected positive and negative responsive lines with their parents at PIN 11 (a.) and PIN 12 (b.)

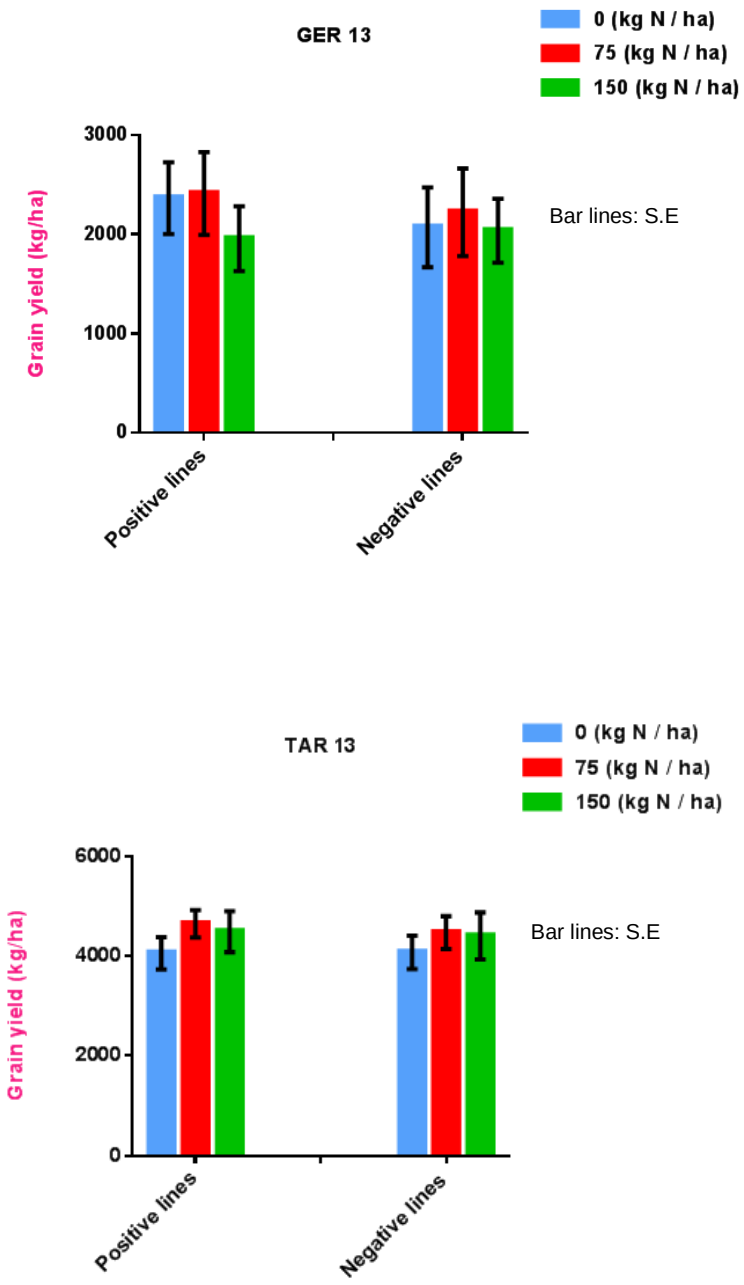


Fig. 2 The average grain yield (kg ha^{-1}) of selected positive and negative responsive lines at GER 13 and TAR 13

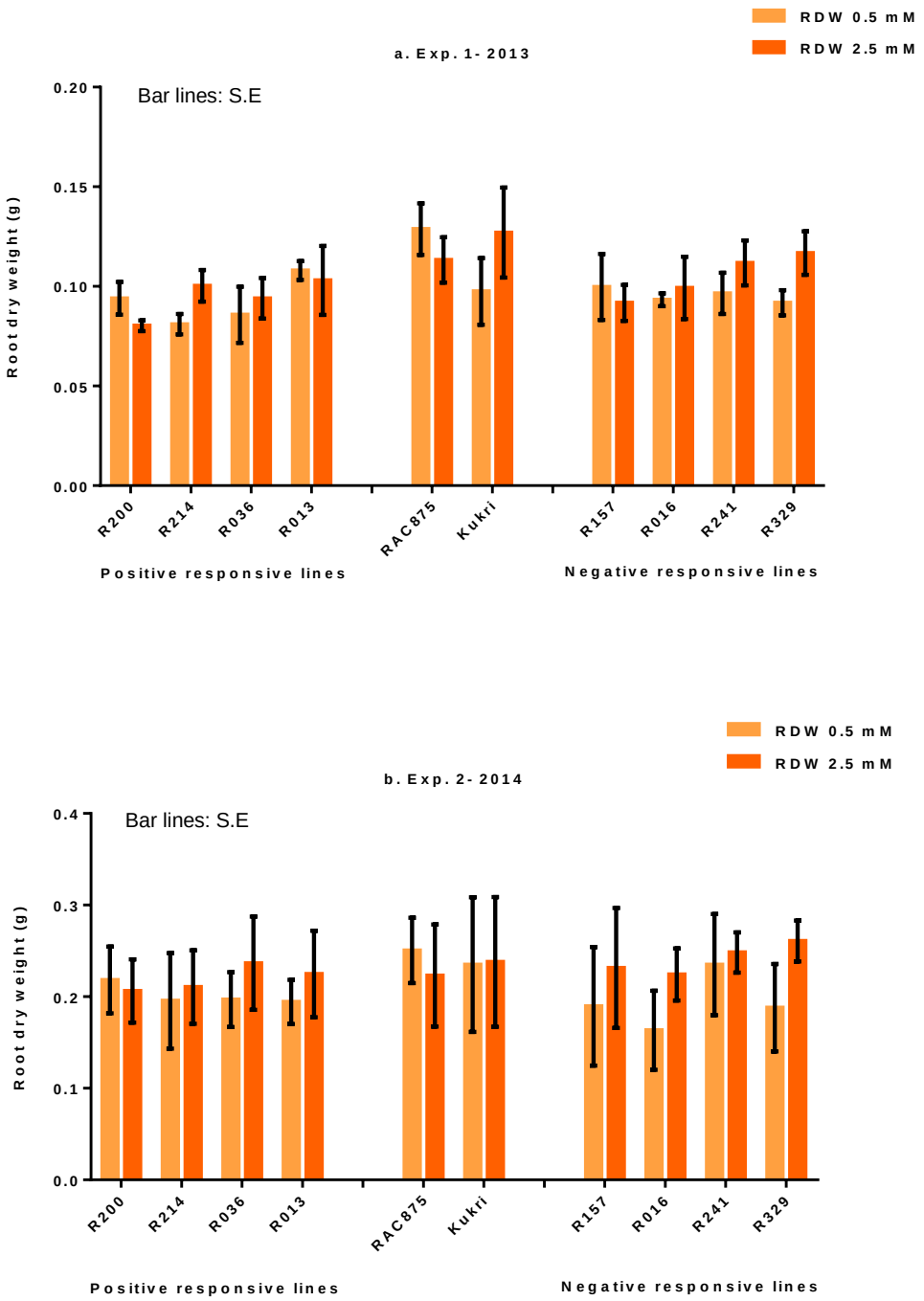


Fig. 3 Root dry weight (RDW) measured in positive and negative responsive lines with their parents under hydroponics experiments; a. Exp. 1- 2013 and b. Exp. 2- 2014

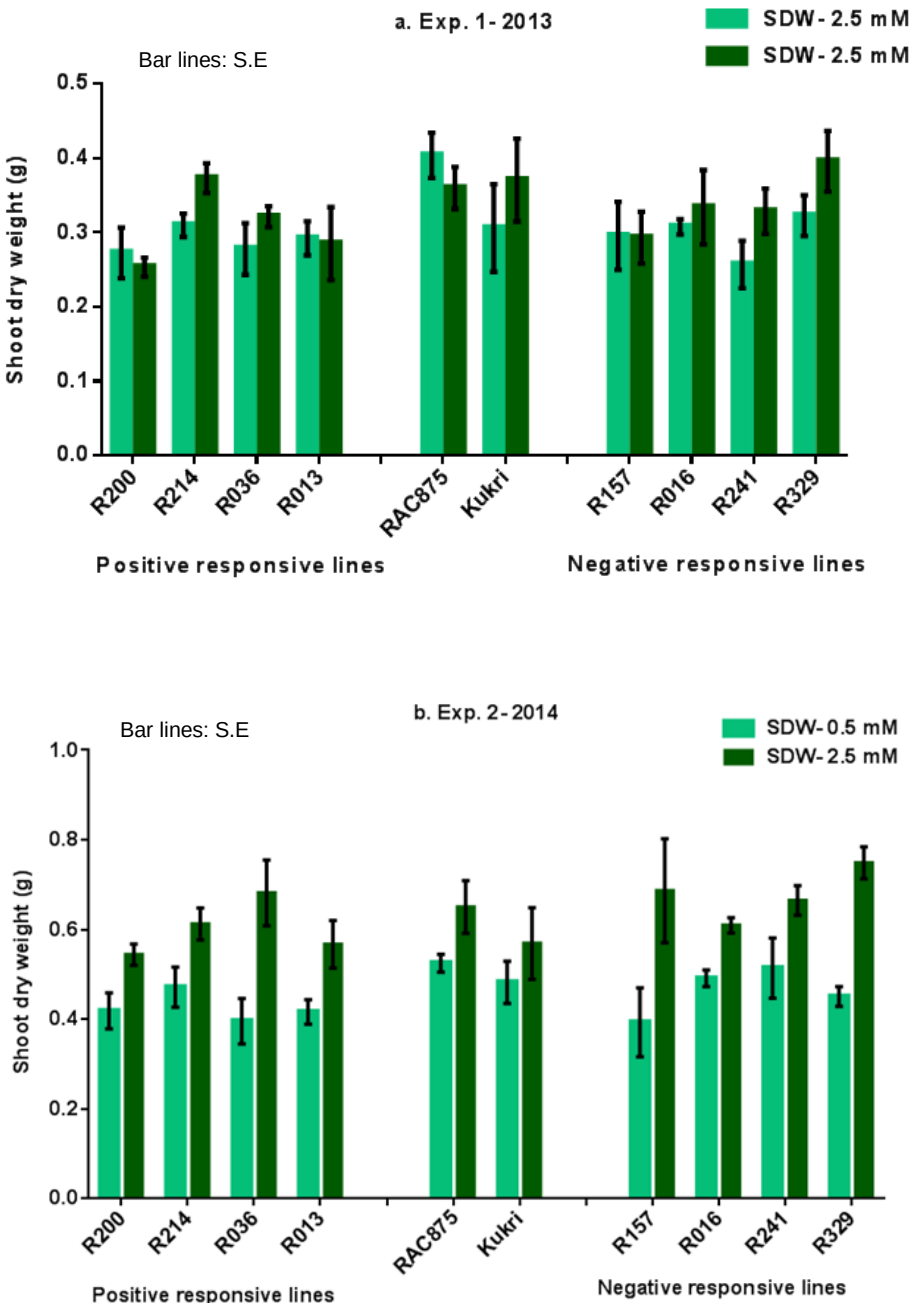


Fig. 4 Shoot dry weight (SDW) measured in positive and negative responsive lines with their parents under hydroponics experiments; a. Exp. 1- 2013 and b. Exp. 2- 2014

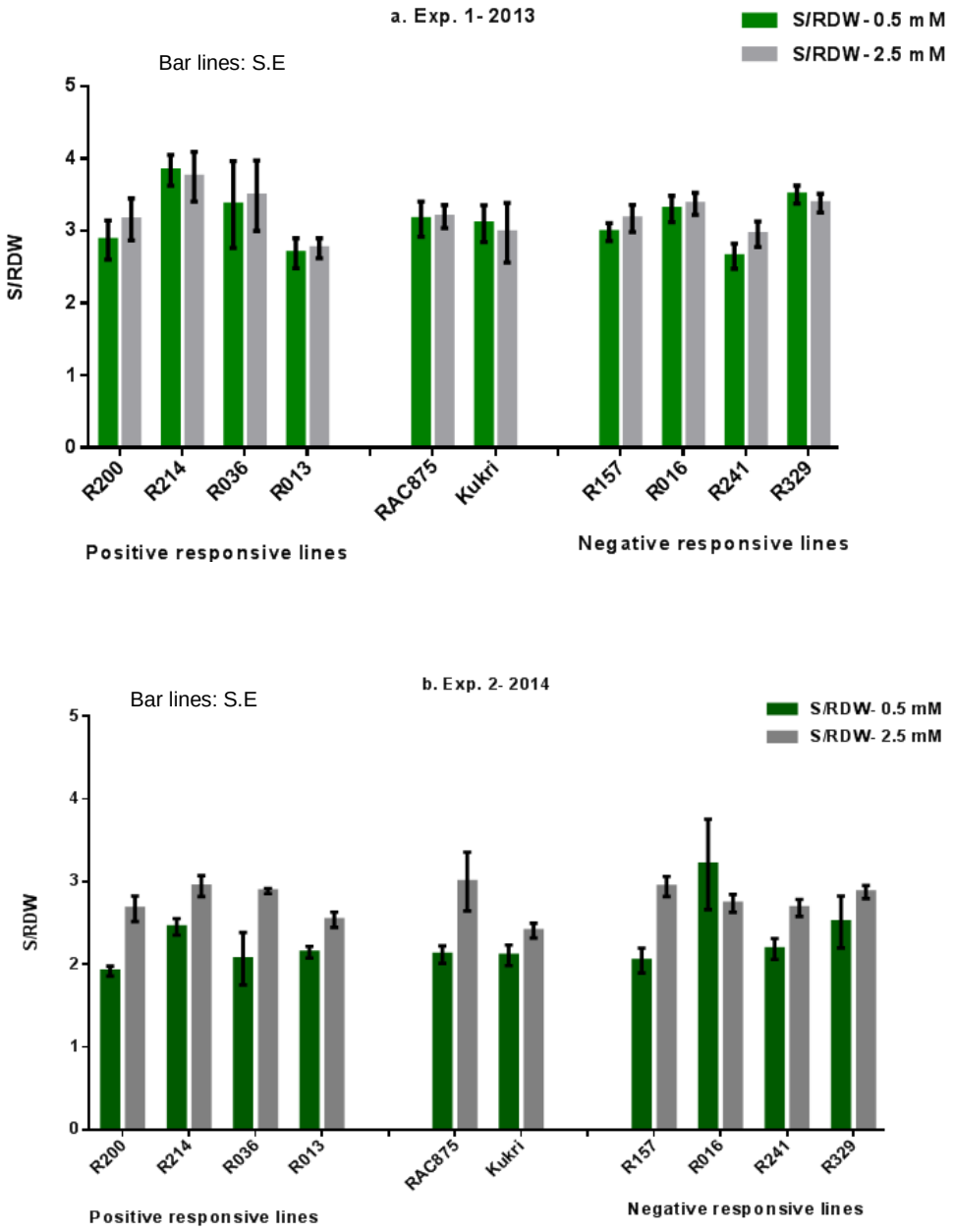


Fig. 5 Shoot to root dry weight ratio (S/RDW) measured in positive and negative responsive lines with their parents under hydroponics experiments; a. Exp. 1- 2013 and b. Exp. 2- 2014

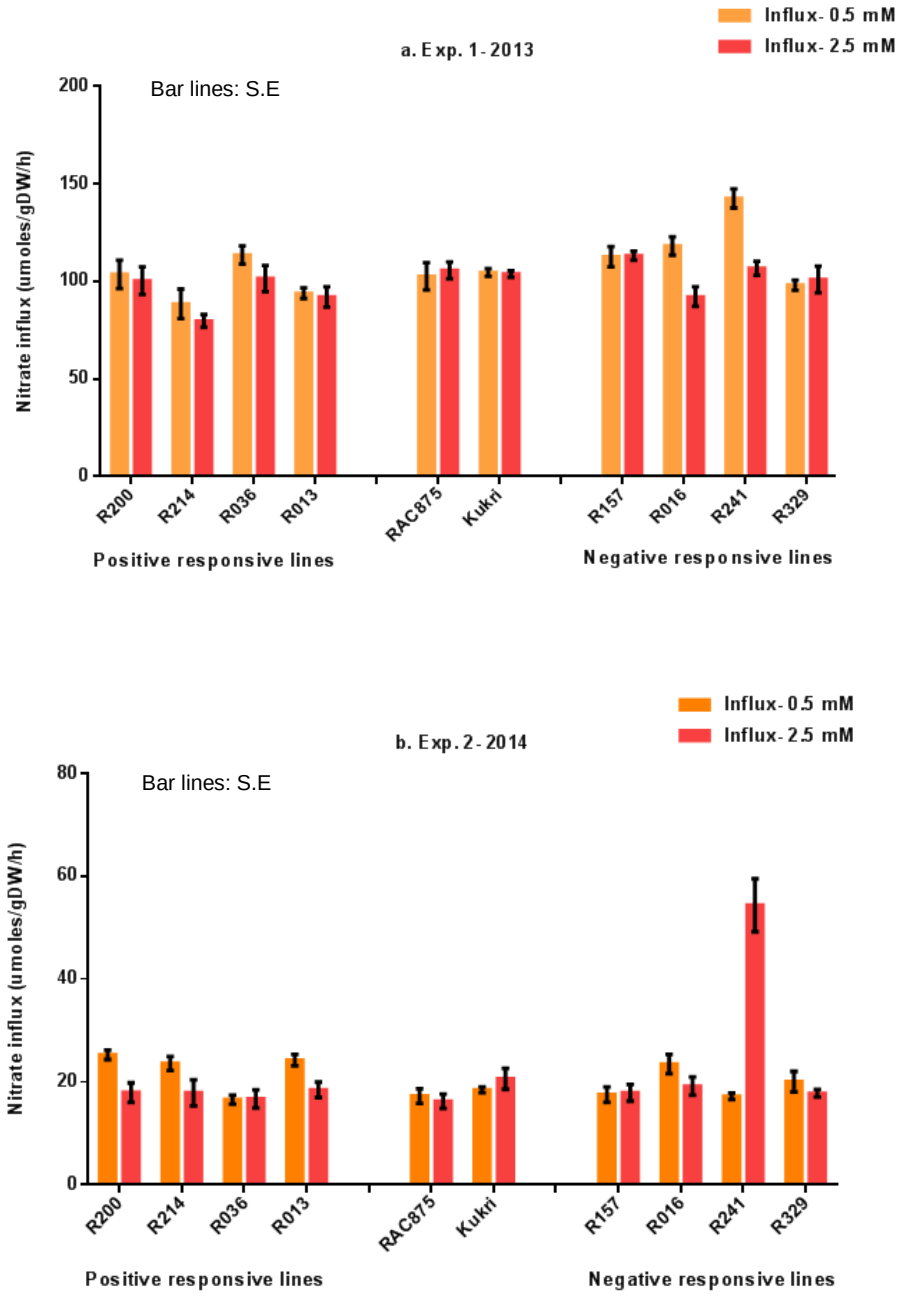


Fig. 6 Nitrate influx of the positive and negative responsive lines with their parents under hydroponics experiments; a. Exp. 1- 2013 and b. Exp. 2- 2014

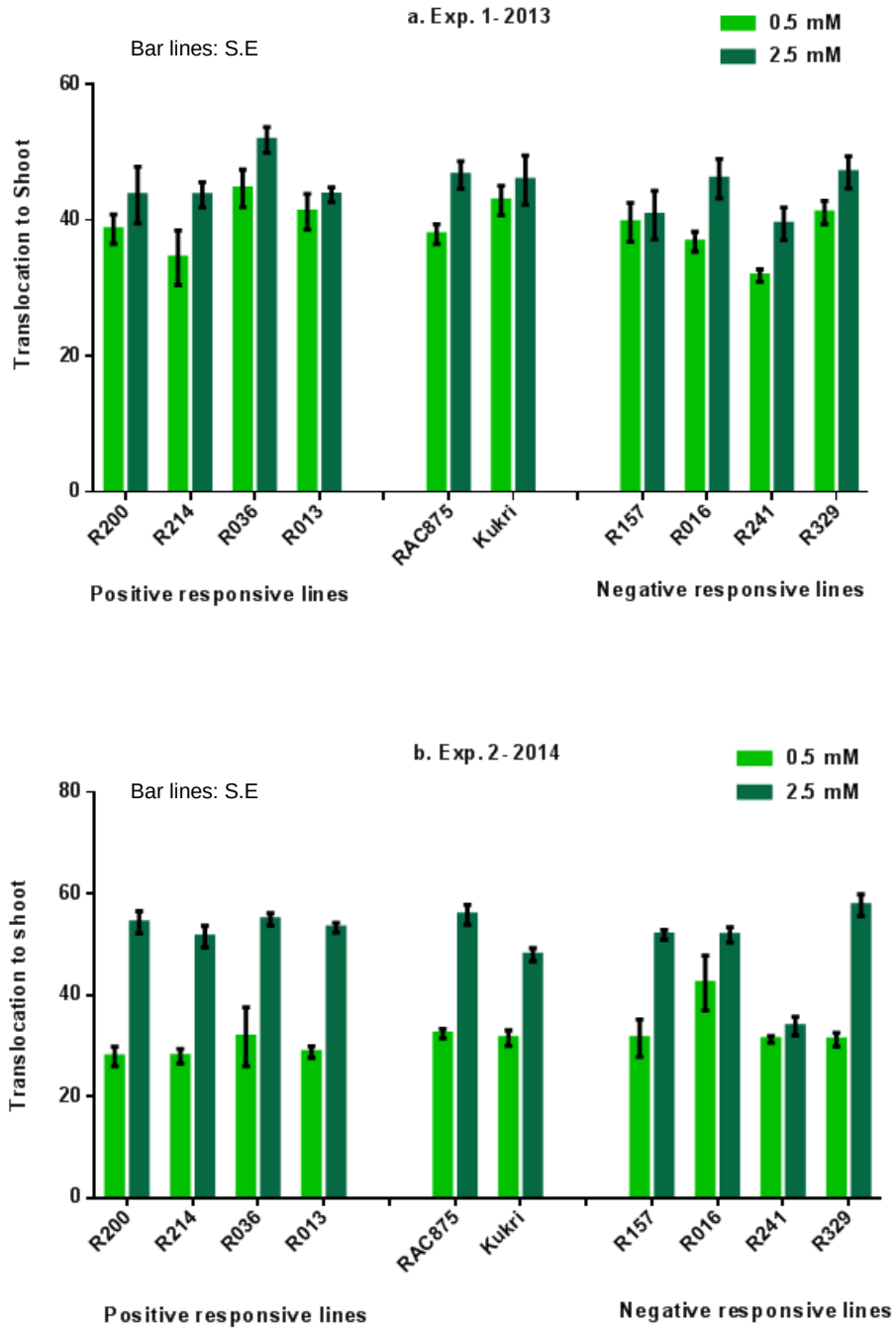


Fig. 7 Translocation to shoot measured in positive and negative responsive lines with their parents under hydroponics experiments; a. Exp. 1- 2013 and b. Exp. 2- 2014

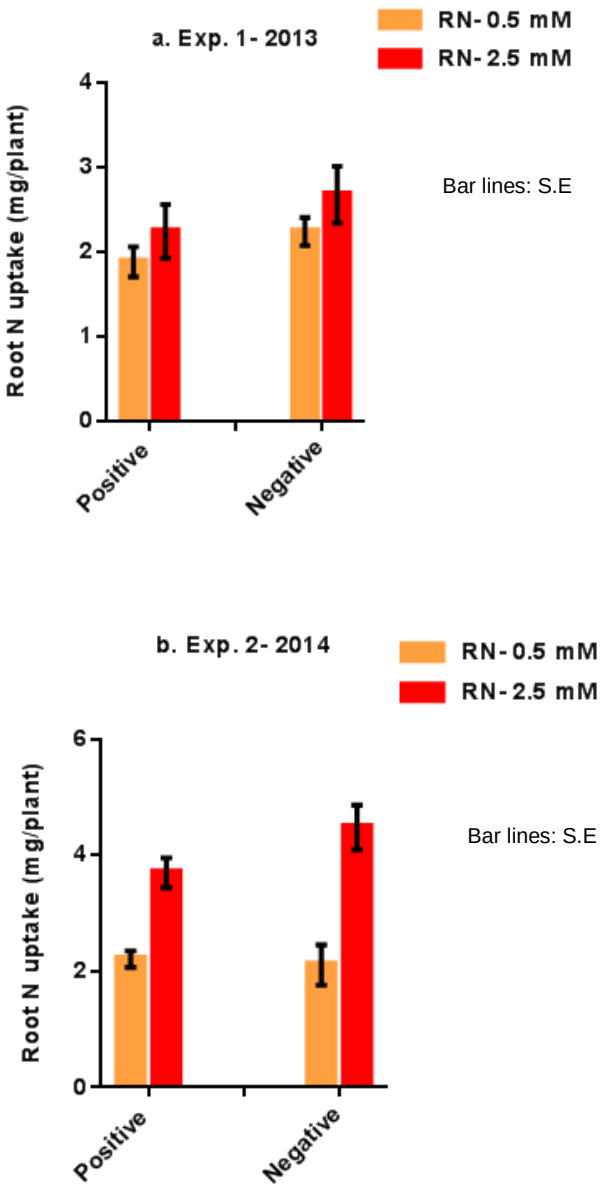


Fig. 8 Root nitrogen (N) uptake of the positive and negative responsive wheat lines under hydroponics experiments; a. Exp. 1- 2013 and b. Exp. 2- 2014

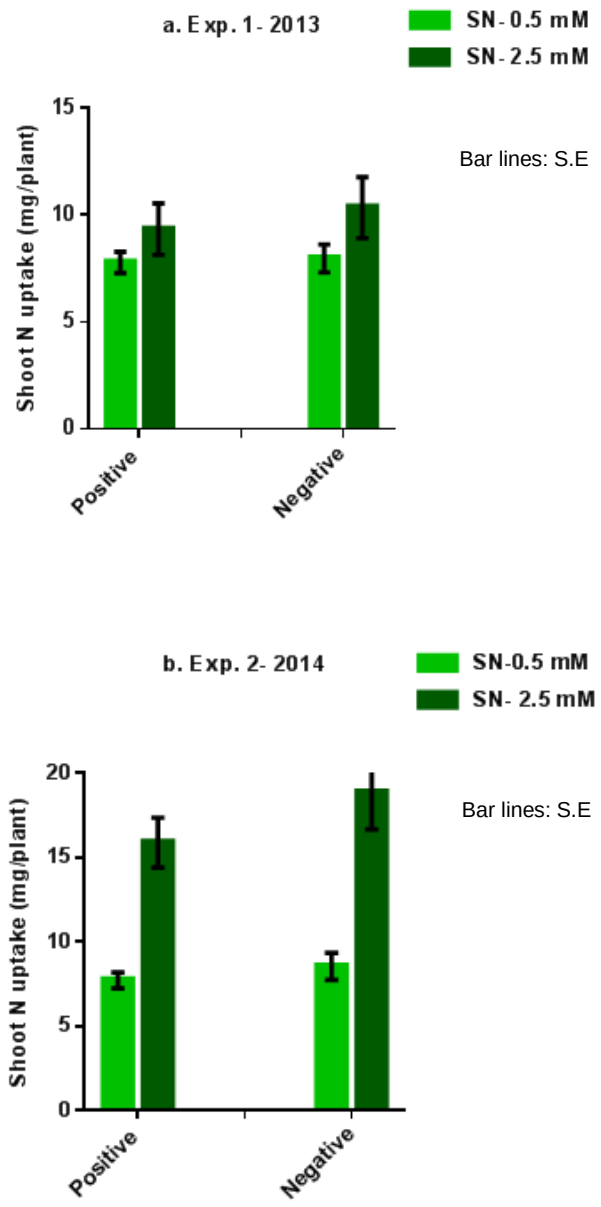


Fig. 9 Shoot nitrogen (N) uptake of the positive and negative responsive wheat lines under hydroponics experiments; a. Exp. 1- 2013 and b. Exp. 2- 2014

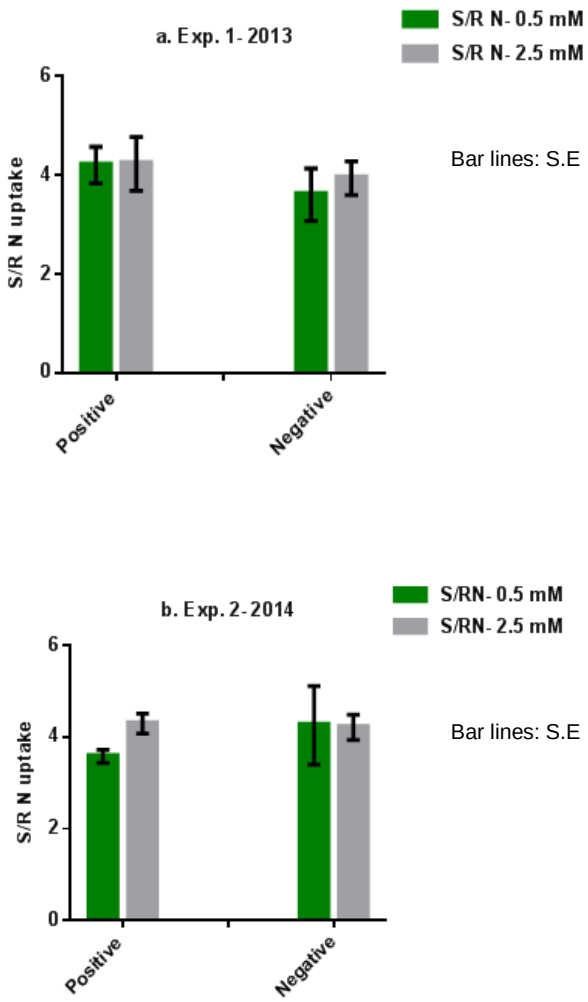


Fig. 10 Shoot to root nitrogen (N) uptake ratio (S/RN) measured in wheat genotypes under hydroponics experiments; a. Exp. 1- 2013 and b. Exp. 2- 2014

Chapter 7

General Discussion

Most previous studies of nitrogen use efficiency (NUE) in wheat have been undertaken in European or high-yielding environments where crop biomass is strongly correlated with yield. In the dry and low yield environments of Southern Australia, high biomass can be a liability late in the growing season where water is limiting and high transpiration rates from leaves can lead to the early onset of severe droughts tress. The starting hypothesis for the work described in this thesis was that well adapted lines in Australia had been selected for the ability to manage their response to nitrogen (N) so that they are able to use available N while limiting early growth. The genetic basis of NUE and N response was studied in popular Australian wheat genotypes and in a doubled haploid (DH) population of RAC875 and Kukri.

In the first research component, the findings from the NUE field trials in South Australia were discussed. Genetic variation for grain yield (GY) in response to differing N regimes was identified in the set of local elite genotypes. NUE was calculated on the basis of GY relative to applied N and showed mixed results depending on the combination of genotypes and environmental conditions, indicating the complexity of this trait. However, the observed variation allowed us to select and rank the genotypes that showed consistent NUE and N response across trials. The contrasting germplasm can be used to develop new populations and support further NUE studies in Australia. The highly ranked cultivars can be suggested to the Australian breeding programs as advantageous for N related traits.

This study also evaluated the genetic basis of GY and protein-related traits using a mapping population from the RAC875/Kukri cross (Chapters 4 and 5). Nitrogen responsiveness and efficiency was used to rank DH lines and dissect the genetic basis for these traits. Quantitative Trait Loci (QTL) analysis detected significant genome regions controlling the traits of interest and also stable QTL, detected across multiple environments and treatments, for GY and responsive GY (RGY) on chromosomes 1A, 1B, 2A, 3D, 7A and 7B. This result represented one of the main findings of this research and will enable breeders to select these loci in the future. Co-located QTL and possibly the same genes for both

production traits, yield and protein content with a negative correlation, were identified on chromosomes 2A, 3A, 7A and 7D. This demonstrated the potential to break the negative link between GY and high protein allowing breeders to improve both traits simultaneously. In addition, these results can be further used for fine mapping to identify genes underlying GY and protein-related traits. Overall, the detected QTL showed that Kukri alleles tended to lead to increases in the traits interest. It should be highlighted that the effect of flowering gene(s) was considered in the analysis of the trial results. However, these parents were originally selected for their differing response to drought stress with Kukri seen as the drought sensitive genotype. The different trend for N response emphasises the importance of studying these traits under conditions where both water and NUE are considered at the same time. Hence, including drought measurements in the study of NUE is recommended for future studies.

Further, the physiological basis of NUE and N response in the selected genotypes was evaluated under controlled growth conditions. Contrasting DH lines and their parents were studied for N uptake and related traits in a hydroponics system at the early of growth stage. However, there was no clear association for N response between field and controlled trials in this study. More studies will be needed to analyse this mismatch in particular to assess the main growth time point where the differences between positive and negative responsive lines are expressed.

To conclude, the work described in this thesis was undertaken to study and dissect N response in Australian wheat genotypes using integrated physiological and genetic approaches. The results showed that particularly at sites with significant G×N, the response to N fertilisation at full N level was higher than that to mid N level. A high level of N fertiliser is needed to achieve high GY in Australian environments. However, there is a need for more studies and evaluation of NUE in wheat under various environmental conditions and N application levels.

References (General Introduction and Literature Review)

Agrama H, Zakaria A, Said F, Tuinstra M (1999) Identification of quantitative trait loci for nitrogen use efficiency in maize Mol Breed 5:187-195

An D, Su J, Liu Q, Zhu Y, Tong Y, Li J, Jing R, Li B, Li Z (2006) Mapping QTLs for nitrogen uptake in relation to the early growth of wheat (*Triticum aestivum* L.) Plant Soil 284:73-84

Bancal P (2009) Decorrelating source and sink determinism of nitrogen remobilization during grain filling in wheat Ann Bot 103:1315-1324

Basso B, Cammarano D, Troccoli A, Chen D, Ritchie JT (2010) Long-term wheat response to nitrogen in a rainfed Mediterranean environment: Field data and simulation analysis Eur J Agron 33:132-138

Bertin P, Gallais A (2001) Genetic variation for nitrogen use efficiency in a set of recombinant inbred lines II - QTL detection and coincidences Maydica 46:53-68

Blackmer T, Schepers J (1994) Techniques for monitoring crop nitrogen status in corn Commun Soil Sci Plant Anal 25:1791-1800

Blum A (1988) Plant breeding for stress environments.

Bogard M, Jourdan M, Allard V, Martre P, Perretant MR, Ravel C, Heumez E, Orford S, Snape J, Griffiths S (2011) Anthesis date mainly explained correlations between post-anthesis leaf senescence, grain yield, and grain protein concentration in a winter wheat population segregating for flowering time QTLs J Exp Bot 62:3621-3636

Bordes J, Ravel C, Jaubertie J, Duperrier B, Gardet O, Heumez E, Pissavy A, Charmet G, Le Gouis J, Balfourier F (2013) Genomic regions associated with the nitrogen limitation response revealed in a global wheat core collection Theor Appl Genet 126:805-822

Brauer EK, Shelp BJ (2010) Nitrogen use efficiency: re-consideration of the bioengineering approach Botany 88:103-109

Chantret N, Salse J, Sabot F, Rahman S, Bellec A, Laubin B, Dubois I, Dossat C, Sourdille P, Joudrier P, Gautier MF, Cattolico L, Beckert M, Aubourg S, Weissenbach J, Caboche M, Bernard M, Leroy P, Chalhoub B (2005) Molecular basis of evolutionary events that shaped the hardness locus in diploid and polyploid wheat species (Triticum and aegilops) Plant Cell 17:1033-1045

Chardon F, Noël V, Masclaux-Daubresse C (2012) Exploring NUE in crops and in Arabidopsis ideotypes to improve yield and seed quality J Exp Bot 62:2131-2142

Chardon F, Virlon B, Moreau L, Falque M, Joets J, Decousset L, Murigneux A, Charcosset A (2004) Genetic architecture of flowering time in maize as inferred from quantitative trait loci meta-analysis and synteny conservation with the rice genome Genetics 168:2169-2185

Charmet G, Robert N, Branlard G, Linossier L, Martre P, Triboï E (2005) Genetic analysis of dry matter and nitrogen accumulation and protein composition in wheat kernels Theor Appl Genet 111:540-550

Chen ZJ (2007) Genetic and epigenetic mechanisms for gene expression and phenotypic variation in plant polyploids Annu Rev Plant Biol 58:377

Coque M, Gallais A (2006) Genomic regions involved in response to grain yield selection at high and low nitrogen fertilization in maize Theor Appl Genet 112:1205-1220

Coque M, Martin A, Veyrieras J, Hirel B, Gallais A (2008) Genetic variation for N-remobilization and postsilking N-uptake in a set of maize recombinant inbred lines. 3. QTL detection and coincidences Theor Appl Genet 117:729-747

Cormier F, Faure S, Dubreuil P, Heumez E, Beauchêne K, Lafarge S, Praud S, Le Gouis J (2013) A multi-environmental study of recent breeding progress on nitrogen use efficiency in wheat (Triticum aestivum L.) Theor Appl Genet 126:3035-3048

Cox MC, Qualset CO, Rains DW (1985) Genetic-variation for nitrogen assimilation and translocation in wheat .2. Nitrogen assimilation in relation to grain-yield and protein Crop Sci 25:435-440

Crews TE, Peoples MB (2005) Can the synchrony of nitrogen supply and crop demand be improved in legume and fertilizer-based agroecosystems? A review Nutrient Cycling in Agroecosystems 72:101-120

Dhugga KS, Waines J (1989) Analysis of nitrogen accumulation and use in bread and durum wheat Crop Sci 29:1232-1239

Elouafi I, Nachit M, Elsaleh A, Asbati A, Mather D (2000) QTL-mapping of genomic regions controlling gluten strength in durum (*Triticum turgidum* L. var. durum) Durum wheat improvement in the Mediterranean region: New challenges International Centre for Advanced Mediterranean Agronomic Studies, Zaragoza, Spain 40:363-371

Fageria N, Baligar V (2005) Enhancing nitrogen use efficiency in crop plants Adv Agron 88:97-185

Flowers M, Tarleton B, Meijer A, Heiniger R, Weisz R (2003) Field validation of a remote sensing technique for early nitrogen application decisions in wheat Agron J 95:167-176

Fontaine J-X, Ravel C, Pageau K, Heumez E, Dubois F, Hirel B, Le Gouis J (2009) A quantitative genetic study for elucidating the contribution of glutamine synthetase, glutamate dehydrogenase and other nitrogen-related physiological traits to the agronomic performance of common wheat Theor Appl Genet 119:645-662

Gallais A, Coque M (2005) Genetic variation and selection for nitrogen use efficiency in maize: a synthesis Maydica 50:531-547
Gallais A, Coque M, Quilléré I, Prioul JL, Hirel B (2006) Modelling postsilking nitrogen fluxes in maize (Zea mays) using 15N-labelling field experiments New Phytol 172:696-707
Gallais A, Hirel B (2004) An approach to the genetics of nitrogen use efficiency in maize J Exp Bot 55:295-306
Gan Y, Miller P, McConkey B, Zentner R, Stevenson F, McDonald C (2003) Influence of diverse cropping sequences on durum wheat yield and protein in the semiarid Northern Great Plains Agron J 95:245-252
Gasser J, Thorburn M (1972) The growth, composition and nutrient uptake of spring wheat J Agric Sci 78:393-404
Gastal F, Lemaire G (2002) N uptake and distribution in crops: an agronomical and ecophysiological perspective J Exp Bot 53:789-799
Glass AD, Britto DT, Kaiser BN, Kinghorn JR, Kronzucker HJ, Kumar A, Okamoto M, Rawat S, Siddiqi M, Unkles SE (2002) The regulation of nitrate and ammonium transport systems in plants J Exp Bot 53:855-864
Goffinet B, Gerber S (2000) Quantitative trait loci: a meta-analysis Genetics 155:463-473
Good AG, Johnson SJ, De Pauw M, Carroll RT, Savidov N, Vidmar J, Lu Z, Taylor G, Stroeher V (2007) Engineering nitrogen use efficiency with alanine aminotransferase Botany 85:252-262
Good AG, Shrawat AK, Muench DG (2004) Can less yield more? Is reducing nutrient input into the environment compatible with maintaining crop production? Trends Plant Sci 9:597-605
Goulding K (2004) Pathways and losses of fertilizer nitrogen at different scales Agriculture and the Nitrogen Cycle:209-219
Greenwood DJ, Karpinets TV, Zhang K, Bosh-Serra A, Boldrini A, Karawulova L (2008) A unifying concept for the dependence of whole-crop n : P ratio on biomass: Theory and experiment Ann Bot 102:967-977
Gregory P, Crawford D, McGowan M (1979) Nutrient relations of winter wheat Accumulation and distribution of Na, K, Ca, Mg, P, S, and NJ Agric Sci Camb 93:485-494
Groos C, Robert N, Bervas E, Charmet G (2003) Genetic analysis of grain protein-content, grain yield and thousand-kernel weight in bread wheat Theor Appl Genet 106:1032-1040
Guarda G, Padovan S, Delogu G (2004) Grain yield, nitrogen-use efficiency and baking quality of old and modern Italian bread-wheat cultivars grown at different nitrogen levels Eur J Agron 21:181-192
Gulmezoglu N, Aytac Z (2010) Response of grain and protein yields of triticale varieties at different levels of applied nitrogen fertilizer Afr J Agric Res 5:2563-2569
Guo Y, Kong F-m, Xu Y-f, Zhao Y, Liang X, Wang Y-y, An D-g, Li S-s (2012) QTL mapping for seedling traits in wheat grown under varying concentrations of N, P and K nutrients Theor Appl Genet 124:851-865
Habash D, Massiah A, Rong H, Wallsgrove R, Leigh RA (2001) The role of cytosolic glutamine synthetase in wheat Ann Appl Biol 138:83-89
Habash DZ, Bernard S, Schondelmaier J, Weyen J, Quarrie SA (2007) The genetics of nitrogen use in hexaploid wheat: N utilisation, development and yield Theor Appl Genet 114:403-419
Hao QN, Zhou XA, Ai HS, Wang C, Zhou R, Chen SL (2011) Identification of genes associated with nitrogen-use efficiency by genome-wide transcriptional analysis of two soybean genotypes BMC Genomics 12:525
Harper L, Langdale G, Giddens J (1987) Nitrogen cycling in a wheat crop: soil, plant, and aerial nitrogen transport Agron J 79:965-973
Hibberd JM, Sheehy JE, Langdale JA (2008) Using C-4 photosynthesis to increase the yield of rice - rationale and feasibility Curr Opin Plant Biol 11:228-231
Hirel B, Bertin P, Quilleré I, Bourdoncle W, Attagnant C, Dellay C, Gouy A, Cadiou S, Retailliau C, Falque M (2001) Towards a better understanding of the genetic and physiological basis for nitrogen use efficiency in maize Plant Physiol 125:1258-1270
Hirel B, Le Gouis J, Ney B, Gallais A (2007) The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches J Exp Bot 58:2369-2387
Hirel B, Lea PJ (2001) Ammonia assimilation. Plant Nitrogen.
Ikram S, Chardon F (2010) Plant quantitative traits eLS
Joppa L, Du C, HarT GE, Hareland GA (1997) Mapping gene (s) for grain protein in tetraploid wheat (*Triticum turgidum* L.) using a population of recombinant inbred chromosome lines Crop Sci 37:1586-1589
Justes E, Mary B, Meynard JM, Machet JM, Thelierhuche L (1994) Determination of a critical nitrogen dilution curve for winter-wheat crops Ann Bot 74:397-407
Kichey T, Hirel B, Heumez E, Dubois F, Le Gouis J (2007) In winter wheat (*Triticum aestivum* L.), post-anthesis nitrogen uptake and remobilisation to the grain correlates with agronomic traits and nitrogen physiological markers Field Crops Res 102:22-32
Kjaer B, Jensen J (1996) Quantitative trait loci for grain yield and yield components in a cross between a six-rowed and a two-rowed barley Euphytica 90:39-48
Laperche A, Brancourt-Hulmel M, Heumez E, Gardet O, Hanocq E, Devienne-Barret F, Le Gouis J (2007) Using genotype× nitrogen interaction variables to evaluate the QTL involved in wheat tolerance to nitrogen constraints Theor Appl Genet 115:399-415

Laperche A, Devienne-Barret F, Maury O, Le Gouis J, Ney B (2006) A simplified conceptual model of carbon/nitrogen functioning for QTL analysis of winter wheat adaptation to nitrogen deficiency Theor Appl Genet 113:1131-1146
Le Gouis J (2011) Genetic Improvement of Nutrient Use Efficiency in Wheat" in Molecular basis of nutrient use efficiency incrops First edn. John Wiley, Oxford
Le Gouis J, Béghin D, Heumez E, Pluchard P (2000) Genetic differences for nitrogen uptake and nitrogen utilisation efficiencies in winter wheat Eur J Agron 12:163-173
Lea PJ, Azevedo RA (2007) Nitrogen use efficiency. 2. Amino acid metabolism Ann Appl Biol 151:269-275
Lemaire G, Gastal F (2009) Quantifying crop responses to nitrogen deficiency and avenues to improve nitrogen-use efficiency Crop physiology: applications for genetic improvement and agronomy Academic Press, San Diego:171–211
Li J, Zhang Z, Li Y, Wang Q, Zhou Y (2011) QTL consistency and meta-analysis for grain yield components in three generations in maize Theor Appl Genet 122:771-782
Lian X, Xing Y, Yan H, Xu C, Li X, Zhang Q (2005) QTLs for low nitrogen tolerance at seedling stage identified using a recombinant inbred line population derived from an elite rice hybrid Theor Appl Genet 112:85-96
Liao MT, Fillery IRP, Palta JA (2004) Early vigorous growth is a major factor influencing nitrogen uptake in wheat Funct Plant Biol 31:121-129
López-Bellido L, López-Garrido F, Fernández E, Fuentes M, Castillo J (1996) Long-term tillage, crop rotation, and nitrogen fertilizer effects on wheat yield under rainfed Mediterranean conditions Agron J 88:783-791
Lopez-Bellido R, Shepherd C, Barraclough P (2004) Predicting post-anthesis N requirements of bread wheat with a Minolta SPAD meter Eur J Agron 20:313-320
Loudet O, Chaillou S, Merigout P, Talbotec J, Daniel-Vedele F (2003) Quantitative trait loci analysis of nitrogen use efficiency in Arabidopsis Plant Physiol 131:345-358
MacDonald WN, Blom TJ, Tsujita MJ, Shelp BJ (2013) Review: Improving nitrogen use efficiency of potted chrysanthemum: Strategies and benefits Can J Plant Sci 93:1009-1016
MacKown CT, Carver BF (2005) Fall forage biomass and nitrogen composition of winter wheat populations selected from grain-only and dual-purpose environments Crop Sci 45:322-328
Man H, Boriel R, El-Khatib R, Kirby EG (2005) Characterization of transgenic poplar with ectopic expression of pine cytosolic glutamine synthetase under conditions of varying nitrogen availability New Phytol 167:31-39
Masclaux-Daubresse C, Daniel-Vedele F, Dechorgnat J, Chardon F, Gaufichon L, Suzuki A (2010) Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture Ann Bot 105:1141-1157
Masclaux-Daubresse C, Reisdorf-Cren M, Orsel M (2008) Leaf nitrogen remobilisation for plant development and grain filling Plant Biol 10:23-36
McDonald G (1992) Effects of nitrogenous fertilizer on the growth, grain yield and grain protein concentration of wheat Aust J Agric Res 43:949-967
Merah O (2001) Potential importance of water status traits for durum wheat improvement under Mediterranean conditions J Agric Sci 137:139-145
Mickelson S, See D, Meyer FD, Garner JP, Foster CR, Blake TK, Fischer AM (2003) Mapping of QTL associated with nitrogen storage and remobilization in barley (Hordeum vulgare L.) leaves J Exp Bot 54:801-812
Moll RH, Kamprath EJ, Jackson WA (1982) Analysis and interpretation of factors which contribute to efficiency of nitrogen-utilization Agron J 74:562-564
Ortiz-Monasterio R, Sayre K, Rajaram S, McMahon M (1997) Genetic progress in wheat yield and nitrogen use efficiency under four nitrogen rates Crop Sci 37:898-904
Pedersen A, Zhang K, Thorup-Kristensen K, Jensen LS (2010) Modelling diverse root density dynamics and deep nitrogen uptake-A simple approach Plant Soil 326:493-510
Peoples MB, Boyer EW, Goulding KWT, Heffer P, Ochwoh VA, Vanlauwe B, Wood S, Yagi K, Van Cleemput O (2004) Pathways of nitrogen loss and their impacts on human health and the environment. Agriculture and the nitrogen cycle: assessing the impact of fertilizer use on food production and the environment.
Pestsova E, Ganal M, Röder M (2000) Isolation and mapping of microsatellite markers specific for the D genome of bread wheat Genome 43:689-697
Qi Z, Wu Q, Han X, Sun Y, Du X, Liu C, Jiang H, Hu G, Chen Q (2011) Soybean oil content QTL mapping and integrating with meta-analysis method for mining genes Euphytica 179:499-514
Quarrie S, Steed A, Calestani C, Semikhodskii A, Lebreton C, Chinoy C, Steele N, Pljevljakusić D, Waterman E, Weyen J (2005) A high-density genetic map of hexaploid wheat (Triticum aestivum L.) from the cross Chinese Spring× SQ1 and its use to compare QTLs for grain yield across a range of environments Theor Appl Genet 110:865-880

Quraishi UM, Abrouk M, Murat F, Pont C, Foucrier S, Desmaizieres G, Confolent C, Rivière N, Charmet G, Paux E (2011) Cross-genome map based dissection of a nitrogen use efficiency ortho-metaQTL in bread wheat unravels concerted cereal genome evolution Plant J 65:745-756

Rafalski A (2002) Applications of single nucleotide polymorphisms in crop genetics Curr Opin Plant Biol 5:94-100

Raun WR, Johnson GV (1999) Improving nitrogen use efficiency for cereal production Agron J 91:357-363

Reynolds M, Foulkes MJ, Slafer GA, Berry P, Parry MAJ, Snape JW, Angus WJ (2009) Raising yield potential in wheat J Exp Bot 60:1899-1918

Robertson GP, Vitousek PM (2009) Nitrogen in agriculture: Balancing the cost of an essential resource Annu Rev Environ Resour 34:97-125

Sadras VO, Lawson C, Hooper P, McDonald GK (2012) Contribution of summer rainfall and nitrogen to the yield and water use efficiency of wheat in Mediterranean-type environments of South Australia Eur J Agron 36:41-54

Shi J, Li R, Qiu D, Jiang C, Long Y, Morgan C, Bancroft I, Zhao J, Meng J (2009) Unraveling the complex trait of crop yield with quantitative trait loci mapping in Brassica napus Genetics 182:851-861

Shrawat AK, Carroll RT, DePauw M, Taylor GJ, Good AG (2008) Genetic engineering of improved nitrogen use efficiency in rice by the tissue-specific expression of alanine aminotransferase Plant Biotechnol J 6:722-732

Søgaard R, Alsterfjord M, MacAulay N, Zeuthen T (2009) Ammonium ion transport by the AMT/Rh homolog TaAMT1; 1 is stimulated by acidic pH Pflugers Arch 458:733-743

Svoboda P, Haberle J (2006) The effect of nitrogen fertilization on root distribution of winter wheat Plant Soil Environ 52:308-313

Tindall TA, Stark JC, Brooks RH (1995) Irrigated spring wheat response to topdressed nitrogen as predicted by flag leaf nitrogen concentration J Prod Agric 8:46-52

Tucker H (2000) Hormones, mammary growth, and lactation: a 41-year perspective J Dairy Sci 83:874-884

Vansanford DA, Mackown CT (1986) Variation in nitrogen use efficiency among soft red winter-wheat genotypes Theor Appl Genet 72:158-163

Wang D, Xu Z, Zhao J, Wang Y, Yu Z (2011) Excessive nitrogen application decreases grain yield and increases nitrogen loss in a wheat–soil system Acta Agriculturae Scandinavica, Section B-Soil & Plant Science 61:681-692

Wang G, Bronson KF, Thorp KR, Mon J, Badaruddin M (2014) Multiple Leaf Measurements Improve Effectiveness of Chlorophyll Meter for Durum Wheat Nitrogen Management Crop Sci 54:817-826

Xu G, Fan X, Miller AJ (2012) Plant nitrogen assimilation and use efficiency Annu Rev Plant Biol 63:153-182

Xu Y, Wang R, Tong Y, Zhao H, Xie Q, Liu D, Zhang A, Li B, Xu H, An D (2014) Mapping QTLs for yield and nitrogen-related traits in wheat: influence of nitrogen and phosphorus fertilization on QTL expression Theor Appl Genet 127:59-72